(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 December 2000 (14.12.2000)

PCT

(10) International Publication Number
WO 00/75326 A1

(51) International Patent Classification[7]: C12N 15/12, 15/11, C07K 14/47, A61K 38/17, 48/00, A01K 67/027

(21) International Application Number: PCT/US00/40151

(22) International Filing Date: 6 June 2000 (06.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/327,138 7 June 1999 (07.06.1999)

(71) Applicant: CEDARS-SINAI MEDICAL CENTER [—/US]; 8700 Beverly Boulevard, Los Angeles, CA 90048 (US).

(72) Inventors: AUERNHAMMER, Christoph, J.; Sollerbauerweg 7, 81241 Munchen-Pasing (DE). SHLOMO, Melmed; 9437 Cresta Drive, Los Angeles, CA 90035 (US).

(74) Agents: STEINBERG, Nisan, A. et al.; Pretty, Schroeder & Poplawski, Suite 1900, 444 South Flower Street, Los Angeles, CA 90071 (US).

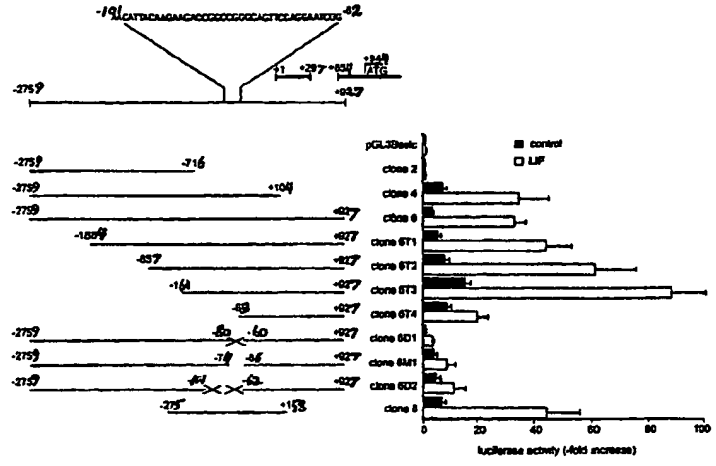
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

[Continued on next page]

(54) Title: SUPPRESSOR OF CYTOKINE SIGNALING (SOCS)-3 PROMOTER AND METHODS FOR ITS USE



(57) Abstract: Disclosed is a nucleic acid construct comprising a murine SOCS-3 promoter sequence having SEQ. ID. NO.:1, or a non-murine homologue thereof, or an operative fragment or derivative. The construct can also contain, operatively linked to the SOCS-3 promoter, a gene encoding any preselected protein, and optionally contains a reporter gene to facilitate detection and/or selection of successfully transfected cells. Also disclosed are a transgenic vertebrate cell containing the nucleic acid construct and transgenic non-human vertebrates comprising such cells. The nucleic acid construct is useful in methods of treating a growth retardation or growth acceleration disorder in a human subject and in a method of treating an autoimmune disease, immune disease, or inflammatory condition in a human subject. A kit for genetically modifying a vertebrate cell includes a polynucleotide comprising the murine SOCS-3 promoter sequence is also disclosed.

WO 00/75326 A1

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

SUPPRESSOR OF CYTOKINE SIGNALING (SOCS)-3 PROMOTER AND METHODS FOR ITS USE

The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of grant DK 50238 awarded by the NIH.

BACKGROUND OF THE INVENTION

Throughout this application various publications are referenced within parentheses. The disclosures of these publications in their entireties are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains.

1. THE FIELD OF THE INVENTION

This invention relates to the medical arts. In particular the present invention relates to the field of cellular signal transduction and to gene therapy.

2. DISCUSSION OF THE RELATED ART

Cytokines are small secreted proteins or factors (5 to 20 kD) that have specific effects on cell-to-cell interactions, intercellular communication, or the behavior of other cells. Cytokines involved in inflammatory diseases are produced by lymphocytes, especially T_H1 and T_H2 lymphocytes, monocytes, intestinal macrophages, granulocytes, epithelial cells, and fibroblasts. (Reviewed in G. Rogler and T. Andus, *Cytokines in inflammatory bowel disease*, World J. Surg. 22(4):382-89 [1998]; H.F. Galley and N.R. Webster, *The immuno-inflammatory cascade*, Br. J. Anaesth. 77:11-16 [1996]). Some cytokines are pro-inflammatory (e.g., tumor necrosis factor [TNF]-α, interleukin [IL]-1(α and β), IL-6, IL-8, IL-12); others are anti-inflammatory (e.g., IL-1 receptor antagonist [IL-1ra], IL-4, IL-10, IL-11, and transforming growth factor [TGF]-β). However, there may be overlap and functional redundancy in their effects under certain inflammatory conditions.

One group of cytokines, the IL-6-type, are also important in the regulation of complex cellular processes such as gene activation, proliferation and differentiation. The IL-6-type

cytokines include IL-6, IL-11, leukemia inhibitory factor (LIF), oncostatin M, ciliary neutrophic factor, and cardiotrophin-1. (Reviewed in P.C. Heinrich *et al., Interleukin-6-type cytokine signaling through the gp130/JAK/STAT pathway*, Biochem. J. 334(Pt 2):297-314 [1998]). The IL-6-type cytokines (also known as the gp130 signaling subunit cytokine family) have in common that signal transduction proceeds through a pathway beginning with ligand binding by type I and type II surface receptors, internalization involving affinity converter/signal transducing subunit gp130, the activation of the Janus family of cytoplasmic tyrosine kinases (e.g., Jak1, Jak2, and Tyk2); this results in the phosphorylation and dimerization of the signal transducers and activators of transcription (STAT)-1 and STAT-3 that activate transcription from promoters having STAT recognition sites. (Heinrich *et al.* [1998]; M. Ernst *et al., Gp130-mediated signal transduction in embryonic stem cells involves activation of Jak and Ras/mitogen-activated protein kinase pathways*, J. Biol. Chem. 271(47):30136-43 [1996]; R. Starr *et al., A family of cytokine-inducible inhibitors of signaling*, Nature 387(6636):917-21 [1997]; T. Hirano *et al.*, Cytokine & Growth Factor Rev. 8:241-52 [1997]; E. Arzt & G.K. Stalla, Neuroimmunomodulation 3:28-34 [1996]; S.J. Haque & B.R.G. Williams, Semin. Oncol. 25 (suppl. 1):14-22 [1998]). This pathway is known as the Jak-STAT signaling cascade.

Several IL-6-type cytokines are important neuro-immuno-endocrine modulators of the hypothalamo-pituitary-adrenal (HPA) axis (Arzt, E. & Stalla [1996]; S. Melmed, Trends Endocrinol. Metab. 8:391-97 [1997]; H.O. Besedovsky, & A. Del Rey, Endo. Rev. 17:64-102 [1996]), which regulates metabolism, including growth, body temperature, water balance, blood sugar, fat metabolism, and sexual and nerve function. For example, LIF is a potent auto-paracrine stimulus of pituitary proopiomelanocortin (POMC) gene expression and adrenocorticotrophic hormone (ACTH) secretion, which stimulates the adrenals to produce additional hormones. Thus, LIF modulates the HPA axis response to various inflammatory and stress stimuli. (Z. Wang et al., Endocrinology 137:2947-53 [1996]; C.J. Auernhammer *et al.*, Endocrinology 139:2201-08 [1998a]). In vitro experiments using human fetal pituitary cells (I. Shimon *et al.*, J. Clin. Invest. 100: 357-63 [1997]) and the corticotroph cell line AtT-20 (S. Akita *et al.*, J. Clin., Invest 95, 1288-1298 [1995]; C. Bousquet *et al.*, J. Biol. Chem 272:10551-57 [1997]), showed a profound and synergistic action of LIF and corticotropin-releasing hormone (CRH) on POMC gene expression and ACTH secretion. LIF is known to induce the Jak-STAT signaling cascade in the corticotroph cells. (C.J.

Auernhammer *et al.*, *Pituitary corticotroph SOCS-3: novel intracellular regulation of leukemia-inhibitory factor-mediated proopiomelanocortin gene expression and adrenocorticotropin secretion*, Mol. Endocrinol. 12(7):954-61 [1998b]; I. Shimon *et al.* [1997]; D.W. Ray *et al.*, *Leukemia inhibitory factor (LIF) stimulates proopiomelanocortin (POMC) expression in a corticotroph cell line. Role of STAT pathway*, J. Clin. Invest. 97(8):1852-59 [1996]; D.W. Ray *et al.*, Ann. N.Y. Acad. Sci. USA 840:162-73 [1998]).

A new family of cytokine-inducible proteins has recently been described that inhibits the Jak-STAT signaling cascade. (E.g., S.E. Nicholson *et al.*, *The SOCS proteins: a new family of negative regulators of signal transduction*, J. Leukoc. Biol. 63(6):665-68 [1998]; R. Starr *et al.*, *SOCS: suppressors of cytokine signaling*, Int. J. Biochem. Cell. Biol. 30(10):1081-85 [1998]). These proteins have been variously termed suppressors of cytokine signaling ("SOCS")(R. Starr *et al.*, *A family of cytokine-inducible inhibitors of signaling*, Nature 387(6636):917-21 [1998]; D.J. Hilton *et al.*, Proc. Natl. Acad. Sci. USA 95:114-19 [1998]), STAT-induced STAT inhibitors (SSI)(T. Naka *et al.*, Nature 387:924-28[1997]; S. Minamoto *et al.*, Biochem. Biophys. Res. Commun 237:79-83 [1997]), cytokine-inducible SH2 containing protein (CIS)(A. Yoshimura *et al.*, EMBO J. 14:2816-26 [1995]; M. Masuhara *et al.*,Biochem. Biophys. Res,. Commun. 239:439-46 [1997]; A, Matsumoto *et al.*, Blood 89:3148-54 [1997]), and Jak binding protein (JAB)(T.A. Endo *et al.*, Nature 387:921-24 [1997]; H. Sakamoto *et al.*, Blood 92:1668-76 [1998]). The SOCS-protein family currently consists of CIS and SOCS-1 through 7. (D.J. Hilton *et al.*[1998]; M.J. Aman & W.J. Leonard, Curr. Biol. 7:R784-R788 [1997]; R. Starr & D.J. Hilton, Int. J. Biochem. Cell Biol. 30:1081-85 [1998]).

SOCS-protein expression is stimulated by various cytokines in a tissue specific manner (R. Starr *et al.*, Nature 387:917-21 [1997]; M.J. Aman & W.J. Leonard [1997]; H. Sakamoto *et al.* [1998]; H.O. Besedovsky, & A. Del Rey [1996]; T.E. Adams *et al.*, J. Biol. Chem. 273:1285-87 [1998]; C. Bjorbaek *et al.*, Mol. Cell 1:619-625 [1998]). The gene expression of SOCS-1/SSI-1/JAB and SOCS-3/SSI-3/CIS-3, referred to herein as SOCS-1 and SOCS-3, are induced by IL-6 and LIF in various tissues (R. Starr *et al.* [1997]; D.J. Hilton *et al.* [1998]; T. Naka *et al.* [1997]; S. Minamoto *et al.* [1997]; M. Masuhara *et al.* [1997]; A. Matsumoto *et al.* [1997]; T.A. Endo *et al.* [1997]). For example, SOCS-3 gene expression is rapidly induced by LIF in the pituitary in vivo, and in corticotroph AtT-20 cells in vitro. (C.J. Auernhammer *et al.* [1998b]).

Both, SOCS-1 and SOCS-3 proteins bind to the JH1 domain of Jak-2 and thereby inhibit IL-6-, IL-11-, or LIF-induced tyrosine phosphorylation activity by Jak-2 of gp 130 and STAT-3. (S. Minamoto *et al.* [1997]; M. Masuhara et al. [1997]; C.J. Auernhammer *et al.* [1998b]). SOCS-3 is induced by growth hormone (GH) in the liver, and inhibits GH-induced Spi 2.1 promoter activity. (T.E. Adams *et al.* [1998]). SOCS-3 inhibits LIF-induced POMC gene expression and ACTH secretion (C.J. Auernhammer *et al.* [1998b]), thus providing an intracellular negative feedback regulation of cytokine-induced activation of the HPA-axis. Hypothalamic SOCS-3 gene expression is stimulated by leptin, and SOCS-3 inhibits leptin-induced signal transduction (C. Bjorbaek *et al.*, Mol. Cell 1:619-625 [1998]), thus suggesting its regulatory role in central leptin resistance.

The structure of SOCS proteins has been described. (e.g., S.E. Nicholson *et al.*, *Mutational analyses of the SOCS proteins suggest a dual domain requirement but distinct mechanisms for inhibition of LIF and IL-6 signal transduction*, EMBO J. 18(2):375-85 (Jan. 1999). Dominant negative STAT-3 mutants, isolated by substitution of a carboxy-terminal tyrosine phosphorylation site Tyr^{705} to Phe^{705} (STAT-3F) or mutation at positions important for DNA binding (STAT-3D) have been recently described (K. Nakajima *et al.*, EMBO J. 15:3651-58 [1996]). Overexpression of these STAT-3 dominant negative mutants in corticotroph AtT-20 cells inhibits LIF-induced POMC gene expression and ACTH secretion. (C. Bousquet & S. Melmed, J. Biol. Chem. 274:10723-30 [1999]). Cytokine-induced gene expression of SOCS-1 has been shown to be inhibited in cells overexpressing dominant negative STAT-3 mutants (T. Naka *et al.* [1997]), but the promoter region of SOCS-1 has not been cloned.

Therefore, there remains a definite need for a promoter sequence capable of regulating expression of preselected proteins, such as SOCS-3 protein, and that can be targeted by gene therapy to treat growth disorders, autoimmune diseases, immune diseases, and inflammatory conditions. This and other features and benefits provided by the present invention will now be described.

SUMMARY OF THE INVENTION

The present invention relates to a nucleic acid construct comprising a murine SOCS-3 promoter sequence, or a non-murine homologue thereof, or an operative fragment or derivative of any of these. The construct can also contain, operatively linked to the SOCS-3

promoter, a DNA sequence encoding a gene for any preselected protein or a gene-specific part of such a DNA sequence, or to a DNA sequence that encodes a preselected gene-specific antisense RNA or a catalytic RNA. A preselected protein that is encoded by the nucleic acid construct can be from an autologous, allogeneic, or xenogeneic source. In addition, the present nucleic acid construct optionally contains a reporter gene to facilitate detection and/or selection of successfully transfected cells. The present nucleic acid construct is particularly useful for linking expression of a desired gene product to physiological processes that are regulated by gp130-mediated signal transduction from IL-6-type cytokines (i.e., cytokines of the gp130 signaling subunit cytokine family), such as IL-6, IL-11, or LIF. For example, when the encoded protein is a SOCS-3 protein, the present nucleic acid can be used to modulate the physiology and/or hormonal secretions of cells of the hypothalamus, pituitary, adrenals, liver, or other tissues, through a negative autoregulatory feedback of SOCS-3 on its own cytokine-induced gene expression.

The present invention also relates to a transgenic vertebrate cell containing the nucleic acid construct of the present invention and to transgenic non-human vertebrates comprising such cells.

The present invention also relates to a method of treating a growth retardation disorder in a human subject. The method involves genetically modifying a GH-responsive or gp130-responsive cell(s) of a human subject having a growth retardation disorder, such as dwarfism, GH deficiency, gonadal dysgenesis, chondrodystrophy, or bone-cartilage dysplasia. The cell(s) are genetically modified using a nucleic acid construct that comprises a SOCS-3 promoter sequence, or operative fragment thereof, operatively linked to a DNA sequence that encodes an RNA that specifically hybridizes to a functional SOCS-3 mRNA. In response to a growth-inducing cytokine, in vivo, the genetically modified cell(s) within the human subject, transcribe an RNA transcript that specifically hybridizes to a functional SOCS-3 mRNA, preventing translation therefrom. This RNA transcript can be an antisense RNA or a catalytic RNA (ribozyme) that cleave the SOCS-3 mRNA. As a consequence, the amount of SOCS-3 protein produced within the genetically modified cell(s) is relatively reduced, and one or more symptoms of the growth retardation disorder in the subject are thereby improved, due to a lessening of SOCS-3-mediated signal suppression within the genetically modified cell(s).

The present invention also relates to a method of treating a growth acceleration

disorder in a human subject. The method involves genetically modifying a GH-responsive or gp130-responsive cell(s) of a human subject having a growth acceleration disorder, such as gigantism, acromegaly, or Cushing's disease. The cell(s) are genetically modified using a nucleic acid construct, comprising a SOCS-3 promoter sequence, or operative fragment thereof, operatively linked to a DNA sequence encoding a SOCS-3 protein, or functional fragment thereof. In response to the growth-inducing cytokine, in vivo, the genetically modified cell(s) produce an enhanced amount of SOCS-3 protein. The symptom(s) of the growth acceleration disorder in the subject are thereby improved, due to enhanced SOCS-3-mediated cytokine signal suppression.

The present invention also relates to a method of treating an autoimmune disease, immune disease, or inflammatory condition in a human subject having a condition, such as Crohn's disease, ulcerative colitis, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, Grave's disease, or a neuroendocrinological response to psychological or physical stress. The method involves genetically modifying a gp130-responsive cell(s), responsive to a pro-inflammatory cytokine, such as IL-6 or LIF. The cell(s) are genetically modified using a nucleic acid construct that includes a SOCS-3 promoter sequence, or operative fragment thereof, operatively linked to a DNA sequence encoding a SOCS-3 protein, or functional fragment thereof. In response to a pro-inflammatory cytokine of the gp130 signaling subunit cytokine family, in vivo, the genetically modified cell(s) produce an enhanced amount of SOCS-3 protein. The symptom(s) of the autoimmune disease, immune disease, or inflammatory condition in the subject are thereby improved, due to a relative increase in SOCS-3-mediated signal suppression.

Alternatively, the SOCS-3 promoter is operatively linked to a DNA sequence encoding a functional anti-inflammatory cytokine of the gp130 signaling subunit cytokine family, such as IL-11, linked to a functional secretory signal. In response to a pro-inflammatory cytokine of the gp130 signaling subunit cytokine family, in vivo, the genetically modified cell(s) produce and secrete an enhanced amount of the anti-inflammatory cytokine. The symptom(s) of the autoimmune disease, immune disease, or inflammatory condition in the subject are thereby improved.

The present invention also relates to a kit for genetically modifying a vertebrate cell. The kit includes a polynucleotide comprising a murine SOCS-3 promoter sequence having SEQ. ID. NO.:1, or an operative fragment or non-murine homologue thereof, or an operative

derivative of any of these. Preferably, the polynucleotide includes, operatively linked to the SOCS-3 promoter, at least one DNA sequence encoding a preselected protein or a gene-specific part of such a DNA sequence, or a DNA encoding a preselected gene-specific antisense RNA or a specific catalytic RNA, as appropriate for a particular application. Optionally, the promoter is linked to a reporter gene for facilitating detection, isolation, or selection of genetically modified cells from unmodified cells. Some embodiments of the kit are configured for use in practicing the present methods of treating a growth retardation or acceleration disorder in a human subject or the present method of treating an autoimmune disease, immune disease, or inflammatory condition in a human subject.

These and other advantages and features of the present invention will be described more fully in a detailed description of the preferred embodiments which follows.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows stimulation of expression from the murine SOCS-3 promoter in corticotroph AtT-20 cells treated with 0.5×10^{-9} M LIF, IL-6, or IL-11 for 60 and 120 min, respectively. Figure 1A shows a Northern blot analysis performed with 25μg total RNA per lane. The upper panel shows SOCS-3 mRNA; the lower panel shows β-actin mRNA. Figure 1B shows luciferase activity in AtT-20 cells transfected with pGL3Basic alone or a -2757/+929 murine SOCS-3 promoter-pGL3Basic construct (clone 6).

Figure 2 shows LIF-induced SOCS-3 promoter activity and gene expression in corticotroph AtT-20 cells overexpressing wild type STAT-3 (AtT-20W) or dominant negative STAT-3 mutants (AtT-20F and AtT-20D), as well as wild type SOCS-3 (AtT-20S) and mock-transfected (AtT-20M); cells were treated with 0.5×10^{-9} M LIF for 45 min. Figures 2A and 2D show Northern blot analysis performed with 15μg total RNA per lane for a representative experiment; upper panel shows SOCS-3 mRNA; lower panel shows β-actin mRNA. Figures 2B and 2E show Northern blot signals for SOCS-3 mRNA analyzed by quantitative densitometry and normalized for β-actin mRNA. Figures 2C and 2F show relative luciferase activity in various cell clones bearing a -2759/+927 murine SOCS-3 promoter-pGL3Basic construct (clone 6).

Figure 3 shows relative luciferase activities in transiently transfected AtT-20 cells bearing different constructs of the genomic 5'-region of murine SOCS-3. Luciferase activity was measured in untreated (filled bars) and LIF-stimulated (unfilled bars) AtT-20 cells.

Crossed lines indicate a deletion of STAT binding elements in Clone 6D1 and 6D2, in between the named nucleotides, A dotted line indicates a mutation of the wild type STAT binding sequence (5'-TTCCAGGAA-3'; SEQ. ID. NO:13) with mutant (5'-ATCGACGAT-3'; SEQ. ID. NO.:14) in clone 6M1.

DETAILED DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The present invention relates to a nucleic acid construct comprising a murine SOCS-3 promoter sequence, or an operative fragment thereof, or a non-murine homologue thereof, or an operative derivative of any of these.

The following nucleotide sequence represents the full length ~3.8-kb genomic 5'-region of the murine SOCS-3 gene (GenBank Accession AF117732). The transcription start site is defined as +1. An untranscribed region extends from nucleotide -2907 to -1, inclusive. A transcribed but untranslated region (exon 1) extends from +1 to +289; exon 2, begins at +854 (exons are underlined), and contains the intronless coding region of SOCS-3 with a translational start site encoded at nt. +944 to +946. (R. Starr *et al.* [1997]). The translation initiation codon ATG (nt. +944 to +946) and a TATA-box (nt. -39 to -34) are indicated in boldface type. Two potential STAT-binding elements (nt. -74 to -66 and nt. -97 to -88) are in boldface and underlined:

-2907 GACGTTCCTA AAAGCATGCA TGTCACCCAG CTTACCCACC CATCTCAGGC CACAGCAGCC

-2847 TGAGAGAGCG GAAGAACACC TGCTGGTCCT GTCCCACCTC TCCTCTTCAA ACAGCCCCAC

-2787 ATCCTCCAGT TTTGCTCTGG GTGGAGCTCC CTGCTGGCCC TGCAGAGGGA AGGCTCTCCT

-2727 AAGCATCATC TATCAGAACG TCTTCAAAAA AAAAAAAAAA AAAAAAAAAG CCTCTCCAGC

-2667 CAGGCTAGCT CTAACACCAT TTCTTCCCCT TCCCCTCTCT CAAATTCACT TATCTTTTTT

-2607 TTTTTTTTTT TTTTTGGATT TTTGAGACAG GGTTTCTCTG TATAGCCCTG GTTGTCCTGG

-2547 AACTCACTTT GTACACCAGC CTGGCCTCGA ACTCAGAGAT CCACCTGCCT CTGCCTCCTG

-2487 AGTGCTGGGA TTAAAGGCGT GCGCCACCAC GCCCGGCTAA ATTCACTTAT CTATTTAATG

-2427 TATATAGGGT ATAGGCTGCC CTTGAACTCA CAAAGATCTG CTTGCTTTGC TTCTGGAATA

-2367 CTAAAGGTGT GTGCTACCAT CACAGGGACC AAGATTTATT TTAATTCTGT ATATGTGTGT

-2307 GTGTGTGTGT GTGTATGGGG GGTGCACATG AGTACAGATT CCCTTGGAGG CCTGGGGTGG

-2247 CTTAGGACTG GGGTTACAAC AGTTGTGACC CATCCTACAT AGGTCCTGGC ACCAACACCC

-2187 CCCCCCCCCC CCCCCGTCTT CCAGAAGTGC AGCAGGTGTT CTTAACTGCT GAGCCAGCAA

-2127 TCCAGCCCCT GACTTCCCTC TCTTACTTAA GAAGCTATCA CAGTGTCTCA CTGGGTCACA

-2067 ATCATGACTA GTCCTTGCTC ATGGCCCACA GCCTCTTCCC CACTGTGGGT TTTGCCCCGC

-2007 AGCTCTGCCG CCCCAGCGCT GCACCCGAGG CCTGACAGAG CCAGGCACGA AGTCAGGGTT

-1947 TGTGGAATGG ATGAATGAAC TTGACTCGTG GCAGAGCATT GTAATTTACA AAGCACTTTC

-1887 CCATCCATTA ACTCCAGGGC TATTTCCTAA GAGTCCTCCC TGTCCTCCAC TGCCCTCGGC

-1827 TCAGAGGCAT ACGGTCAAGG CAGTGGCTGG GGAACACTCC CTGAATGAGA TCAAGGAGGG

-1767 CTTGTTCACA GAGAAAGGGA GAATCCATTT GGGGAGCCTG AGAGTGACTC GAAGGCAAGG

-1707 ACTGGGCCTC ACCTGTGGGA TCTCCATCTG TGAGCATCCG CTCATCAGAC CAGTGTGAGA

-1647 TATTTTAAAT AAGGCCCCTA AGCCTCTTGA CTACTGGAAT TGCCAGGGGC GGGGGACAGA

-1587 TGGGCACCCA TCCCTATTTA ACAGATAACA AGACTGAGTC CAGAGAGGCA GTGCACCTGC

-1527 CCTGGTCTCT CTTAGTTCCT CAGCATCAGT GGAGCAGATT GGACACAGTG GGCCAGAAGG

-1467 GAAGCAGGCA GCCCTCCCTC CTAGCCCAAG CTACTCTGTG TAGTCAGTTT GCCCTCCTAC

-1407 TGGTGTTACA AGAAGCCTGT GGTATCCAAG AGGGCAGGTC AGAAAGCCCA CTGAGAGCAG

-1347 ACACTGTGTG TCACTTAGCT GGTTCTCAGG TGGCTGCCAC TTCCTGCTGC CTGTTGCAAA

-1287 ACTCGACACT AGGCCTTTAT AGATACTCAC GTGACCAGGA GTAAACAACC TTTCACCTCA

-1227 ATCACCTGCT CTTATCAATA CTCCCTCTCC ACCCCACCAT CGGGAAAGTT CAGACACCTT

-1167 AAAACGTAGA GGCAAGAGAG GGTCCATTCT GACACCTCAG CGACTTTCAG GCAGTGGCTG

-1107 AACCCGTTAC AACGCTCTGT GGACAGTCCT CCTAGTCGAC ATTCCTTCTC AGGTTTGACC

-1047 CTGTCCTGGG AAGTGAGGCT TCTCTCTCTG GGTTCCCCAC TCCTGTTCTT GAATAAGGAG

-987 CCCCACAACC TCTTATTCTC TCTATACAGA GCCTGGGAAA CAGCAAAACT CGGCTCGCCT

-927 ACAAGACTCC AGCGCGCCCT CTGGTGGACT CGGGGGACGA GCATGGGATG AGGGTTTCTT

-867 TCCTCTAGCT CCCCCACCGC GCCGAGAGTA CCTGGGCGGA CCCACAGTTC GCCACGCAGG

-807 TTGGGAGGCC CAGATGAGTG ATAAGGTAGT AGTTAGCTGC TCCTCCCACC CCACTCCCCA

-747 AAGGACATCA GCACCCACGT CTGTCACCGA AGAACCAGGC AATGGGCGGA TGAGCTGAGG

-687 CCAGGTAGCT GCTTCTAAGT CAGTGTCTCC TCCACTTCTG GATCTCACAG CTTCATCTTT

-627 TGGACCTGTC TACAGGTAAA TGTCGCGCAT CCCCCTCCTC CACTTCCTAG GTCCCCAGTG

-567 GGCTGGTGGC TGAATGGTCC TACGTCCCTT TTGGTTGGCA CGGGATGCTT GGAACTGTAC

-507 ATGAGGACCT CGGGGTGGCC TGGGTGCAGA GGGAGGGGAG CGTCCCCGCG GGGATCAAAA

-447 GAAAGGGAAG GGGTGCCAGG AGGGAGCCTC TCCCGGCTGG CCTCCTAGAA CTGCCCGCGC

-387 GCTCCCATCG CGACGCCCCC GCCTCTGCCA GAAACCAGCC TTCTTAGAAG GGAGGGGGGG

-327 GAAAGTGTGA ATGAGAAGTT GGGGGCGGAG CGCGCGGGGG AGGGGCCGCT GCCAGGAACG

-267 CTCGGCCAAG GCTGGCGCCG CGCCCGCCGG TCGGGCAGCC TCGCGCCGCG CTTTGTCTCC

-207 CTCTCGGTGA GTCTCGGCGG GTCCTGGAGG CCCCAGCTCC AAGCCCGCCC TCCGCAGCCC

-147 CTCCCTCGCC CTCCGCGCAC AGCCTTTCAG TGCAGAGTAG TGACTAAACA TTACAAGAAG

-87 ACCGGCCGGG CAGTTCCAGG AATCGGGGGG CGGGGCGTAC TGGCCGGGTA AATACCCGCG

-27 CGCGCGGCCT CCGAGGCGGC TCTAACTCTG ACTCTACACT CGCCCGCTCC TACGACCGCT

+34 GTCTCTCCGG GCTCCCGGAC GCCCCCTTCC CGGCCCAGCT CTCCGTCGAG GTCCCTCGCC

+94 CAGGTCCTTT GCCTGATTCG CCCAGGAGTG CGCCTCATCG GCCCGGGGAG CAGCGAAGCC

+154 AGAGGGGGCG CACGCACGGG GAGCCCCTTT GTAGACTTCA CGGCTGCCAA CATCTGGGCG

+214 CAGCGCGAGC CACTGCTGGG CGCCGCCTCG CCTCGGGGAC CATAGGAGGC GCAGCCCCAA
+274 GGCCGGAGAT TTCGCT TCGG GACTAGGTAG GAAGGAGGGG CGCGGTGTGG GGAAGGGTGG
+334 GGGCATCGGT CCAGCTCGGG AGCTTTTCCC GGTTTCTCCT CCCCTTCCCG GGTCATTCCC
+394 GGTAGGGAGG GGACGAGGCA GGGGGCAGAG CGGATGAGAA CCGAAGATCC CTGATTCCCG
+454 TCATACTCAG ACTGGGGCCC TCGGGTTTCT CCTGTCCCCT CTCTCACATA TCTCGGGTTT
+514 GGCACCCCCC TTTTTTCGCC CTCGCCACTG AGGACACCGG ACTGAGAGGC GCCCTGAGCG
+574 TCCCTAGGGC TCTTGTGTCT CTCCCCATCC TGGCCGCGCT CCTGGAGACC CAACTTCCAC
+634 GCGCGAGTTT TCTCTGGGCG TCCTCCTAGG GCGGGCAGGG GAAGAGACTG TCTGGGGTTG
+694 GCCGGCAGTG ACCGAGGACA GTCGAGTTCC GCGAGGTGGC TGGGCCTGAG ACACGGTCTA
+754 AAGCGGGGCA AAGGGGTGCC CCGGGCGCTA GGCGGAGGCT GGAGGGCCGG GCACGCTGGA
+814 GGGTTCCGGG CACTCACGCG CCTCACGCTT TGCTCTCTGC AGCTCCCCGG GATGCGGTAG
+874 CGGCCGCTGT GCGGAGGCCG CGAAGCAGCT GCAGCCACCG CCGCGCAGAT CCACGCTGGC
+934 TCCGTGCGCC ATGGTCACCC ACAGCAAGTT TCCCGCCGCC GGGATGAGCC GCCCCCTGGA
+994 CACCAGCCTG CGCCTCAAGA CCTTCAGCTC CAAAAGCGAG // (SEQ. ID. NO.:1)

A preferred embodiment of the SOCS-3 promoter of the present invention is a DNA fragment with the sequence of nt. -2907 to +1033, inclusive (SEQ. ID. NO.:1). Other preferred embodiments of the SOCS-3 promoter include any operative fragment of SEQ. ID. NO.:1 or non-murine homologue thereof, or an operative derivative of any of these.

Preferred examples of an operative fragment include the -2759 to +104 fragment (SEQ. ID. NO.:2); the -2759 to +927 fragment (SEQ. ID. NO.:3); the -1864 to +927 fragment (SEQ. ID. NO.:4); the -857 to +927 fragment (SEQ. ID. NO.:5); the -63 to +927 fragment (SEQ. ID. NO.:6); the -97 to +927 fragment (SEQ. ID. NO.:7); the -97 to +104 fragment (SEQ. ID. NO.:8); the -87 to +927 fragment (SEQ. ID. NO.:9); the -87 to +104 fragment (SEQ. ID. NO.:10); and the -275 to +158 fragment (SEQ. ID. NO.:11). A most preferred example is the -161 to +927 fragment (SEQ. ID. NO.:12).

Non-murine homologues include any SOCS-3 promoter sequence of non-murine origin that functions in a vertebrate cell type of interest.

Another preferred embodiment of a SOCS-3 promoter is an operative derivative of SEQ. ID. NO:1, or of any operative fragment of SEQ. ID. NO.:1 or non-murine homologue thereof, having the translational start site (the ATG in bold at nt. +944 to +946 of the murine sequence above) changed to ATT, or changed to a codon sequence, other than ATT, that is also not recognized as a translational start site; another preferred SOCS-3 promoter is a

derivative of SEQ. ID. NO.:1 with the codon of the first translational start site deleted altogether. Other operative derivatives include SOCS-3 promoter sequences containing a mutation, polymorphism, or variant allele with respect to any nucleotide position of SEQ. ID. NO.:1 that does not fully eliminate promoter activity, for example, a deletion of nt. -101 to -62, or a deletion of nt. -80 to -60, or a mutation of nt. -74 to -66. The skilled practitioner is aware of suitable methods for site-directed mutagenesis, e.g., the method of Deng and Nickoloff (W.P. Deng and J.A. Nickoloff, Analyt. Biochem.200:81-88 [1992]), and commercial site-directed mutagenesis kits are available, for example the Transformer® site-directed mutagenesis kit (Clontech).

The murine SOCS-3 gene promoter contains a pair of STAT binding elements $TT(N_5)AA$, separated by 14 nucleotides, at nt. -74 to -66 and at nt. -97 to -88. In this respect, the murine SOCS-3 promoter is structurally similar to the human CIS gene promoter, which contains two functionally important pairs of STAT binding elements and is upregulated by a STAT-5 dependent pathway. (A. Yoshimura *et al.* [1995]; A. Matsumoto *et al.* [1997]; F. Verdier *et al.*, Mol. Cell. Biol. 18:5852-60 [1998]). However, for activity from the present SOCS-3 promoter, only the STAT binding element at -74 to -66 is essential for optimal operability.

In a preferred embodiment, the SOCS-3 promoter is operatively linked to a DNA having a DNA sequence encoding any preselected protein or series of preselected proteins. For purposes of the present invention, "operatively linked" means that the promoter sequence, is located directly upstream from the coding sequence and that both sequences are oriented in a 5' to 3' manner, forming a transcriptional unit, such that transcription could take place in vitro in the presence of all essential enzymes, transcription factors, co-factors, activators, and reactants, under favorable physical conditions, e.g., suitable pH and temperature. This does not mean that, in any particular cell, conditions will favor transcription.

These DNA sequence encoding a preselected protein(s), or a gene-specific part, are derived from the genome of any eukaryotic organism, prokaryotic organism, or virus, and can be autologous, allogeneic, or xenogeneic with respect to the host cell. DNA sequences having a "normal" form of a gene, or a desirable allele thereof are useful in genetic therapy to compensate for endogenous production of defective protein(s) or the underexpression or

overexpression of normal protein(s). In some embodiments, natural variant alleles of a gene are used, or novel genetic modification(s) are artificially induced in the DNA sequence encoding the preselected protein. Variant alleles or mutations are not limited to single nucleotide polymorphisms (SNPs), but also include deletions, insertions, inversions, translocations, transitions, tranversions, or repeats. Mutations or variations are artificially induced in the DNA sequence encoding the preselected protein by a number of techniques, all of which are well known in the art. Alternatively, the DNA sequence linked to the SOCS-3 promoter encodes a gene-specific antisense RNA, such as an antisense RNA that specifically hybridizes to SOCS-3 mRNA, preventing translation therefrom. In another embodiment, the DNA sequence encodes a catalytic RNA, such as a "hairpin" or "hammerhead" ribozyme, that specifically hybridizes to a predetermined mRNA of interest and cleaves it, thereby preventing any further translation therefrom.

Most preferably, transcription of the DNA sequence from the SOCS-3 promoter results in RNA transcript that is biologically active in the cell or organism of interest, for example, as mRNA that is translated into functional protein(s); or as antisense RNA that specifically hybidizes with a functional mRNA of interest, for example a SOCS-3 mRNA, and thus prevents its translation to protein; or as catalytic RNA that specifically hybridizes with and cleaves a predetermined mRNA of interest.

In one embodiment, the preselected protein is a SOCS-3 protein, or a functional fragment thereof. Transcription of the DNA sequence encoding the SOCS-3 protein produces mRNA transcript, which is translated into SOCS-3 protein, or a functional fragment thereof. Thus one benefit of the present invention is that the nucleic acid can be used in a genetic therapy to correct clinical disorders derived from defective negative regulation of cytokine signal transduction in GH-responsive or gp130-responsive cells. Such defective negative regulation can result from, but need not result from, endogenous underexpression of functional SOCS-3 protein, which protein inhibits in an autocrine manner the cytokine-induced Jak-STAT cascade and SOCS-3 protein synthesis itself. But an unmodulated cellular response to GH and IL-6-type cytokine signaling caused by a defect in any of various components of the cellular signal transduction mechanism can also be negatively regulated using the present nucleic acid construct containing a DNA sequence encoding a SOCS-3 protein, or a functional fragment thereof.

In another embodiment, the DNA sequence operatively linked to the SOCS-3 promoter, encodes a SOCS-3-specific nucleotide sequence, transcription of which results in the production of RNA transcript in an antisense orientation that can hybridize to SOCS-3-encoding mRNA to prevent synthesis of SOCS-3 protein. In another embodiment, SOCS-3-specific sequences are included in a DNA sequence that encodes a catalytic RNA that specifically hybridizes to SOCS-3 mRNA. These embodiments are beneficially applied to genetic therapy to correct clinical disorders derived from negative overregulation of cytokine signal transduction in GH-responsive or gp130-responsive cells.

Other preferred embodiments of the present nucleic acid construct also include, operatively linked to the SOCS-3 promoter, a DNA sequence encoding a reporter protein for facilitating the detection or selection of cells containing the present nucleic acid construct and expressing from the SOCS-3 promoter. Preferably, but not necessarily, the reporter gene encodes a fluorescent protein. Fluorescent proteins include green fluorescent protein (or enhanced green fluorescent protein), yellow fluorescent protein, blue fluorescent protein, a phycobiliprotein, such as phycoerythrin or phycocyanin, or any other protein which fluoresces under suitable wave-lengths of light. Another reporter gene suitable for some applications is a gene encoding a protein that can enzymatically lead to the emission of light from a substrate(s); for purposes of the present invention, such a protein is a "light-emitting protein." For example, a light-emitting protein includes proteins such as luciferase or apoaequorin.

The DNA of animal cells is subject to methylation at the 5' carbon position of the cytidine bases of CpG dinucleotides. Unmethylated CpGs are found preferentially in transcriptionally active chromatin. (T. Naveh-Many *et al.*, *Active gene sequences are undermethylated*, Proc. Natl. Acad. Sci. USA 78:4246-50 [1981]). Hypermethylation is associated with transcriptional repression. (R. Holliday, *The inheritance of epigenetic defects*, Science 238:163-70 [1987]). Since some vertebrate cell types of interest may silence expression from the present SOCS-3 promoter sequence by methylation, the skilled practitioner is aware that suitable insulator elements are employed to prevent methylation of the promoter sequence. Preferably, this is done by flanking the transcriptional unit of the promoter sequence and included gene(s) with insulator elements. For example, by including double copies of the 1.2 kb chicken β-globin insulator element 5' to the SOCS-3 promoter

sequence and 3' to the operatively linked gene(s) in the present DNA construct, methylation will be substantially prevented at CG dinucleotide sites within the SOCS-3 promoter sequence and thus expression therefrom occurs. (M.J. Pikaart *et al.*, *Loss of transcriptional activity of a transgene is accompanied by DNA methylation and histone deacetylation and is prevented by insulators*, Genes Dev. 12:2852-62 [1998]; Chung *et al.*, *DNA sequence which acts as a chromatin insulator element to protect expressed genes from cis-acting regulatory sequences in mammalian cells*, U.S. Patent No. 5,610,053).

The present invention also relates to a transgenic vertebrate cell containing the nucleic acid construct of the present invention, regardless of the method by which the construct was introduced into the cell. The present cell is a growth hormone (GH)-responsive or gp130-responsive cell, for example, a cell that specifically binds any IL-6-type cytokine (i.e., binds a cytokine of the gp130 signaling subunit cytokine family). Embodiments include pituitary cells, hypothalamic cells, adrenal cells, intestinal cells, kidney cells, liver cells (e.g., hepatocytes), immune-competent cells, or bone-forming cells, such as chondrocytes. In one embodiment, the present cell is a corticotroph cell, but the cell may also be an intestinal epithelial cell, a lymphocyte, a somatotroph, a lactotroph, or a gonadotroph cell. For some in vitro applications, for example with a wide variety of non-murine cells, inhibitors of histone deacetylation and DNA methylation, such as trichostatin A or sodium butyrate, can be included in the culture medium to prevent possible silencing of expression from the SOCS-3 promoter. (M.J. Pikaart *et al.* [1998]).

The transgenic cells of the present invention are detected, isolated or selected from non-transgenic cells with the aid of, for example, a flow-activated cell sorter (FACS), set at the appropriate wavelength(s). Alternatively, the transgenic cells are detected, isolated or selected manually from non-transgenic cells using conventional microscopic technology.

In particular applications involving a transgenic cell that expresses additional xenogeneic genes from any promoter, this expression may be linked to a reporter gene that encodes a different fluorescent or light-emitting protein from the reporter gene linked to the SOCS-3 promoter. Thus, multiple reporters fluorescing or emitting at different wavelengths can be chosen and cell selections based on the expression of multiple traits can be made.

The present invention also relates to transgenic non-human vertebrates comprising such cells, for example, non-human primates, mice, rats, rabbits, gerbils, hamsters, canines,

felines or other non-human mammals. Other vertebrates include birds such as chickens, turkeys, ducks, ostriches, emus, geese, guinea fowl, doves, quail, rare and ornamental birds, and the like. Broadly speaking, a "transgenic" vertebrate is one that has had foreign DNA permanently introduced into its cells. The foreign gene(s) which (have) been introduced into the animal's cells is (are) called a "transgene(s)." The present invention is applicable to the production of transgenic vertebrates containing xenogeneic, i.e., exogenous, transgenic genetic material, or material from a different species, including biologically functional genetic material, in its native, undisturbed form. In other embodiments, the genetic material is "allogeneic" genetic material, obtained from different strains of the same species, for example, from animals having a "normal" form of a gene, or a desirable allele thereof.

Gene delivery is by any suitable method including in vivo and vitro gene delivery methods. (E.g., D.T. Curiel *et al.*, U.S. Patent Nos. 5,521,291 and 5,547,932). Typically, gene delivery involves exposing a cell to a gene delivery mixture that includes preselected genetic material together with an appropriate vector, mixed, for example, with an effective amount of lipid transfecting agent (lipofection). The amount of each component of the mixture is chosen so that gene delivery to a specific species of cell is optimized. Such optimization requires no more than routine experimentation. The ratio of DNA to lipid is broad, preferably about 1:1, although other proportions may also be utilized depending on the type of lipid agent and the DNA utilized. This proportion is not crucial. Other well known gene delivery methods include electroporation or chemical methods. (E.g., M. Ostresh, *No barriers to entry: transfection tools get biomolecules in the door*, The Scientist 13(11):21-23 (1999).

"Transfecting agent", as utilized herein, means a composition of matter added to the genetic material for enhancing the uptake of exogenous DNA segment(s) into a vertebrate cell. The enhancement is measured relative to the uptake in the absence of the transfecting agent. Examples of transfecting agents include adenovirus-transferrin-polylysine-DNA complexes. These complexes generally augment the uptake of DNA into the cell and reduce its breakdown during its passage through the cytoplasm to the nucleus of the cell.

Other preferred transfecting agents include Lipofectin®, DMRIE C, Cellfectin® or Lipofectamine (Life Technologies), LipoTAXI (Stratagene), Superfect or Effectene (Qiagen). Although these are not as efficient gene delivery (or transfecting) agents as viral transfecting

agents, they have the advantage that they facilitate stable integration of xenogeneic DNA sequence into the vertebrate genome, without size restrictions commonly associated with virus-derived transfecting agents. A virus, or transfecting fragment thereof, can be used to facilitate the delivery of the genetic material into the cell. Examples of suitable viruses include adenoviruses, adeno-associated viruses, retroviruses such as human immune-deficiency virus, other lentiviruses, such as Moloney murine leukemia virus and the retrovirus vector derived from Moloney virus called vesicular-stomatitis-virus-glycoprotein (VSV-G)-Moloney murine leukemia virus, mumps virus, and transfecting fragments of any of these viruses, and other viral DNA segments that facilitate the uptake of the desired DNA segment by, and release into, the cytoplasm of cells and mixtures thereof. All of the above viruses may require modification to render them non-pathogenic or less antigenic. Other known vector systems, however, are also useful.

The present invention also relates to a method of treating a growth retardation disorder in a human subject, especially in a child or adolescent. The method involves genetically modifying a GH-responsive or gp130-responsive cell of a human subject having a growth retardation disorder, typically resulting in short stature, such as, but not limited to, dwarfism, GH deficiency, gonadal dysgenesis, chondrodystrophy, bone-cartilage dysplasia, or an idiopathic condition of severe short stature. Typically, the cell is a pituitary, adrenal, hypothalamic, liver, immune-competent, or bone-forming cell that is responsive to a growth-inducing cytokine in a paracrine manner. Examples include hepatocyte, lymphocyte, lymphocyte, chondrocyte, corticotroph, somatotroph, lactotroph, or gonadotroph cells, or cells derived from a pituitary tumor, adrenal tumor, hypothalamic tumor, liver tumor, or bone tumor.

The cell(s) are genetically modified by any suitable method, in vivo or in vitro, for example by transfection or transduction, using a nucleic acid construct of the present invention, comprising a SOCS-3 promoter sequence, or operative fragment thereof, operatively linked, in a transcriptional unit, to a DNA sequence encoding an RNA that specifically hybridizes to a functional SOCS-3 mRNA, i.e., a SOCS-3-specific antisense RNA. In response to the presence of a growth-inducing cytokine, in vivo, the cell transcribes, from the transcriptional unit, RNA transcript that hybridizes to SOCS-3 mRNA, preventing translation therefrom. This RNA transcript can be an antisense RNA or a catalytic

RNA (ribozyme) that cleave the SOCS-3 mRNA. As a consequence, the amount of SOCS-3 protein produced within the genetically modified cell(s) is reduced relative to unmodified cells of the same kind, and one or more symptoms of the growth retardation disorder in the human subject are thereby improved, due to a lessening of SOCS-3-mediated suppression of gp130-mediated signal transduction from growth-inducing cytokines, such as GH, within the genetically modified cell(s).

The present invention also relates to a method of treating a growth acceleration disorder in a human subject. The method involves genetically modifying a GH-responsive or gp130-responsive cell from a tissue of a human subject having a growth acceleration disorder, resulting in greater than normal enlargement of one or more parts of the body, such as, but not limited to, gigantism, acromegaly, Cushing's disease, or an idiopathic condition resulting in abnormal, non-edemic enlargement of bones, or facial or other soft tissue features. Typically, the cell is a pituitary, adrenal, hypothalamic, liver, immune-competent or bone-forming cell that is responsive to a growth-inducing cytokine in a paracrine manner. Examples include hepatocyte, lymphocyte, chondrocyte, corticotroph, somatotroph, lactotroph, or gonadotroph cells, or cells derived from a pituitary tumor, adrenal tumor, hypothalamic tumor, liver tumor, or bone tumor.

The cell(s) are genetically modified by any suitable method, in vivo or in vitro, for example by transfection or transduction, using a nucleic acid construct, in accordance with the present invention, comprising a SOCS-3 promoter sequence, or operative fragment thereof, operatively linked, in a transcriptional unit, to a DNA sequence encoding a SOCS-3 protein, or functional fragment thereof. In response to a growth-inducing hormone or cytokine, in vivo, SOCS-3 mRNA transcript is transcribed from the transcriptional unit, resulting in translation of SOCS-3 message to SOCS-3 protein. The amount of SOCS-3 protein produced is thereby enhanced in the genetically modified cell(s) in response to the presence of a growth-inducing cytokine, such as GH or a cytokine of the gp130 signaling subunit family, compared to the amount in unmodified cells of the same kind. The symptom(s) of the growth acceleration disorder in the subject are thereby improved, due to increased SOCS-3-mediated cytokine signal suppression within the genetically modified cell(s). Thus, for example, in pituitary corticotroph cells, ACTH secretion is suppressed by increased levels of SOCS-3, ultimately leading to less production of glucocorticoid hormones

by the adrenals and ameliorating symptoms of Cushing's disease. Similarly, the effects of excess GH, as for example in acromegaly, are moderated in accordance with the present method.

The present invention also relates to a method of treating an autoimmune disease, immune disease, or inflammatory condition in a human subject. Such diseases or conditions include, but are not limited to, Crohn's disease, ulcerative colitis, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, Grave's disease, allergic or anaphylactic reactions, or neuroendocrinological responses to psychological or physical stress. The method involves genetically modifying a cell(s) from the subject that is gp130-responsive, i.e., responsive to at least one pro-inflammatory cytokine, such as IL-6, LIF, or any other pro-inflammatory cytokine for which signal transduction is gp130-mediated. Typically, the cell is a pituitary, adrenal, hypothalamic, liver, intestinal, nerve, kidney, immune-competent, or bone-forming cell that is responsive to a pro-inflammatory cytokine in a paracrine manner. Examples include hepatocyte, lymphocyte, chondrocyte, neuron, intestinal epithelial, corticotroph, somatotroph, lactotroph, or gonadotroph cells.

The cell(s) are genetically modified by any suitable method, in vivo or in vitro, for example by transfection or transduction, using a nucleic acid construct comprising a SOCS-3 promoter sequence, or an operative fragment thereof, operatively linked, in a transcriptional unit, to a DNA sequence encoding a SOCS-3 protein, or functional fragment thereof. In response to an inflammatory cytokine of the gp130 signaling subunit family, in vivo, SOCS-3 mRNA transcript is transcribed from the transcriptional unit, resulting in translation of SOCS-3 message to SOCS-3 protein. The amount of SOCS-3 protein produced is thereby enhanced in the genetically modified cell(s) in response to the presence of an inflammatory cytokine of the gp130 signaling subunit family, compared to the amount in unmodified cells of the same kind. One or more symptoms of the autoimmune disease, immune disease, or inflammatory condition in the subject are thereby improved, due to a relative increase in SOCS-3-mediated signal suppression.

In another embodiment, the nucleic acid construct that is used in the method comprises a SOCS-3 promoter sequence, or operative fragment thereof, operatively linked, in a transcriptional unit, to a DNA sequence encoding a functional anti-inflammatory cytokine of the gp130 signaling subunit cytokine family, such as IL-11, linked to a functional

secretory signal. In response to the presence of a pro-inflammatory cytokine of the gp130 signaling subunit cytokine family, in vivo, the anti-inflammatory cytokine is produced and secreted by the modified cell(s), which has both paracrine and autocrine effects that improve One or more symptoms of the autoimmune disease, immune disease, or inflammatory condition in the subject.

In some embodiments of the present methods, gene delivery is done in vitro, and the cell(s) is first obtained from a tissue of the human subject by any suitable biopsy method, for example percutaneous biopsy, laparoscopic biopsy, or stereotactic cranial biopsy. Gene delivery is accomplished in vitro, and the genetically modified cell(s) are then re-implanted within the tissue of the human subject.

The nucleic acid construct that is used in the present methods optionally contains a reporter gene for convenient detection, isolation or selection of transgenic cells expressing from the SOCS-3 promoter as described herein. For particular applications, other DNA sequences encoding other preselected proteins are optionally linked to the SOCS-3 promoter, making their expression inducible by IL-6-type cytokines and gp130-mediated signal transduction.

The present invention also relates to a kit for genetically modifying a vertebrate cell. The kit is a ready assemblage of materials or components for facilitating the genetic modification of a vertebrate cell. The kit includes a polynucleotide comprising a murine SOCS-3 promoter sequence having SEQ. ID. NO.:1, or an operative fragment or non-murine homologue thereof, or an operative derivative of any of these, as described herein with respect to the nucleic acid construct of the present invention. Preferably the polynucleotide includes a transcriptional unit that contains the SOCS-3 promoter, operatively linked to at least one DNA sequence encoding a preselected protein or to a gene-specific part thereof, such as a SOCS-3 protein, or a functional fragment thereof, and/or a reporter gene for facilitating detection, isolation, or selection of genetically modified cells from unmodified cells. The DNA sequence encoding the preselected protein can be in a sense or antisense orientation as appropriate for a particular application. Some embodiments of the kit are configured for use in practicing the present methods of treating a growth retardation or acceleration disorder in a human subject or the present method of treating an autoimmune disease, immune disease,

or inflammatory condition in a human subject.

The kit optionally contains a suitable transfecting agent, as described above. The kit includes instructions for using the materials or components effectively. The materials or components assembled in the kit are provided to the practitioner stored in any convenient and suitable way that preserves their operability and utility. For example the components can be in dissolved, dehydrated, or lyophilized form; they can be provided at room, refrigerated or frozen temperatures.

The foregoing descriptions of the nucleic acid constructs, transgenic cells, transgenic vertebrates, methods, and kits of the present invention are illustrative and by no means exhaustive. The invention will now be described in greater detail by reference to the following non-limiting examples.

EXAMPLES

Example 1: Materials and Methods

Materials. Recombinant murine LIF, IL-6, and IL-11 were purchased from R&D Systems (Minneapolis, MN). Mouse liver Marathon-Ready® cDNA, Advantage® -GC cDNA polymerase, mouse GenomeWalker® Kit, and Advantage® -GC genomic polymerase were from Clontech (Palo Alto, CA). Maxiscript® T7 polymerase kit and ribonuclease protection kit RPA-II® were from Ambion (Austin, TX). Polyclonal STAT-1 p84/p91 (M-22) and STAT-3 (H-190) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse genomic DNA, Erase-a Base® system, pGL3 Basic and pSV-β-galactosidase vector were from Promega (Madison, WI). TOPO-TA® PCR2.1 vector was from Invitrogen (Carlsbad, CA).

Cell Culture. Cell culture of AtT-20/D16v-F2 cells was performed as described (C.J. Auernhammer *et al.* [1998b]; C. J. Auernhammer *et al.* [1998a]). Individual clones of AtT-20 cells, overexpressing SOCS-3 (AtT-20S), mock-transfected (AtT-20M), wild type STAT-3 (AtT-20W) or dominant negative STAT-3 mutants (AtT-20 F and AtT-20D), were isolated after stable transfection. (C.J. Auernhammer *et al.* [1998b]; C. Bousquet & S. Melmed, J. Biol. Chem. 274:10723-30 [1998]). From each group, three separate individual clones with high stable overexpression of the respective construct were selected with G418 (1 mg/mL)

for the experiments.

Northern blot analysis. Northern blot analysis was performed as described (C.J. Auernhammer *et al.*[1998b]; C. J. Auernhammer *et al.* [1998a]). To detect endogenous SOCS-3 mRNA in AtT-20S cells, without hybridization to exogenous SOCS-3 mRNA derived from stable overexpression of SOCS-3, a probe spanning exon 1 and the untranslated 5' region of exon 2 as used. Otherwise, the previously described (C.J. Auernhammer *et al.* [1998b]; C. J. Auernhammer & S. Melmed, Endocrinology 140:1559 [1999]) murine SOCS-3 probe spanning most of the coding region of SOCS-3 was used.

5'-Rapid Amplification of cDNA Ends (RACE) and RNase protection Assay. 5'-RACE was performed with a pre-made, adaptor-ligated Marathon-Ready® double stranded cDNA derived from pooled BALB/c mouse liver (A. Chenchik *et al.*, Biotechnol. 3: 526-34 [1996]) and Advantage®-GC cDNA polymerase using gene-specific primary and nested antisense primers 5'-CAGTAGAATCCGCTCTCCTGCAGCTTG-3' (SEQ. ID. NO.:15) and 5'-CTCGCTTTTGGAGCTGAAGGTCTTGAG-3' (SEQ. ID. NO.:16). Products were cloned into PCR2.1 vector, and multiple single clones sequenced.

RNase protection assay was performed with RPA-II® kit, following the manufacturer's recommendations. A fragment spanning nucleotides +158 to -275 was cloned into PCR2.1 vector; the plasmid was linearized with *Bam*HI, and a ^{32}P-UTP labeled antisense probe was generated with T7 polymerase.

PCR-based characterization of the 5'-genomic region. The 5'-genomic region of SOCS-3 was cloned using a PCR-based technique (P.D. Siebert *et al.*, Nucleic Acids Res. 23:1087-88 [1995]) with pre-made adaptor-ligated genomic DNA fragments, derived from ICR Swiss mice, as provided by the Genomewalk® kit. PCR and subsequent nested PCR were performed by automatic hot-start as touchdown-PCR using Advantage®-GC genomic Polymerase and gene specific antisense primers 5'-CAGTAGAATCCGCTCTCCTGCAGCTTG-3' (SEQ. ID. NO.:15) and 5'-CTCGCTTTTGGAGCTGAAGGTCTTGAG-3' (SEQ. ID. NO.:16). Further genomic walks

in the 5' direction were performed with gene specific antisense primers 5'-CTTCCTACCTAGTCCCGAAGCGAAATC-3'(SEQ.ID.NO.:17),5'-CAGATGTTGGCAGCCGTGAAGTCTAC-3'(SEQ.ID.NO.:18), 5'-GCGGGCGAGTGTAGAGTCAGAGTTAGAG-3'(SEQ.ID.NO.:19),and 5'-CGATTCCTGGAACTGCCCGGCCGGTCTTC-3'(SEQ.ID.NO.:20), as well as 5'-CTCAGTGGGCTTTCTGACCTGCCCTCTTG-3' (SEQ.ID.NO.:21) and 5'-GACTACACAGAGTAGCTTGGGCTAGGAG-3'(SEQ.ID.NO.:22). Products were cloned into PCR2.1, and single clones were sequenced.

Different Constructs of the 5' Genomic Region of SOCS-3. 3'-Truncated forms of the full-length 3.7-kb construct in pGL3Basic vector (clone 6) were generated by PCR from genomic DNA and subsequent cloning as described above.

5'-Truncated forms of clone 6 were generated using Erase-a-Base® kit, following the manufacturer's recommendations. Briefly, the 3.7-kb full-length construct of the 5' genomic region of SOCS-3 in pGL3Basic vector was digested with *Sst*I and *Nhe*I, followed by unidirectional digestion with exonuclease III (S. Henikoff, Gene 28:351-59 [1984]) and subsequent re-ligation.

Mutated forms of clone 6 were generated by overlap extension PCR (A. Aiyar *et al.*, Methods Mol. Biol. 57:177-91 [1996]) with Pfu polymerase and 5% DMSO, by using external sense primer 5'-CATCGCGACGCCCCCGCCTCT-3' (SEQ.ID.NO.:23) and antisense primer 5'-GAAACCCGAGGGCCCCAGTCTG-3'(SEQ.ID.NO.:24) with exclusive restriction sites for *Nru*I or *Apa*I, respectively. Internal mutagenizing primers caused deletions of nucleotides -80 to -60 and -101 to -62, respectively. Similarly, the STAT binding element region at -74 to -66 was mutated. Gel-purified PCR-products and the original template were digested with *Nru*I and *Apa*I, fragments were purified, and the mutated fragments were re-ligated into the original 3.7-kb construct in pGL3Basic vector. Each construct was verified by sequencing.

Luciferase Assay. For transient transfection experiments, 2 x 10^5 cells were plated in 6-well plates, incubated for 24 hours, and transfected using Lipofectamine-re and 0.5 μg

of constructs in pGL3Basic vector, and 1.0 μg pSV-β-galactosidase. Transfected cells were first incubated for 24 hours in serum-free DMEM, followed by 6 hours of cytokine treatment and subsequent measurement of luciferase activity. In experiments comparing overexpressing dominant negative STAT-3 mutants or wild type SOCS-3, treatment with LIF was for 45 minutes.

In experiments using different promoter constructs, transfection efficiency was verified by measurement of β-galactosidase activity.

Electromobility shift assay. Nuclear extracts of AtT-20 cells and electromobility shift assay (EMSA) were performed as described (P.D. Siebert *et al.*, Nucleic Acids Res. 23:1087-88 [1995]). Briefly, AtT-20 cells were grown to 80% confluence and were serum-deprived for 24 hours before treatment with 10^{-9} M LIF, followed by cell lysis and preparation of nuclear extracts. For the EMSA, 20-μg nuclear extracts were preincubated for 15 minutes at room temperature in 20 μL binding buffer (10 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA, 1mM DTT, 0.1% NP-40, 5% glycerol, 1 mg/mL BSA, pH 7.5) with 1 μg of poly(dI-dC). A ^{32}P-labeled double stranded oligonucleotide, corresponding to nucleotide sequence -77 to -57 of the SOCS-3 promoter (5'-$^{-77}$CAGTTCCAGGAATCGGGGGGC^{-57}-3)(SEQ.ID.NO.:25), was used as a probe (60,000 cpm, 5 fmol per reaction) and added to each sample and binding reaction, performed at room temperature for 20 min. In competition experiments, 100-fold molar excess of unlabeled double stranded competitor oligonucleotides were added to the preincubation reaction with the double stranded oligonucleotide corresponding to nucleotide sequence -77 to -57 of the SOCS-3 promoter, this same oligonucleotide mutated at positions -74, -71, -69, and -66 (underlined) (5'-$^{-77}$CAGATCGACGATTCGGGGGGC^{-57}-3)(SEQ.ID.NO.:26), or the AP-2 recognition site oligonucleotide 5'-GATCGAACTGACCGCCCGCCGCCCGT-3' (SEQ.ID.NO.:27). For supershift experiments 2 μg polyclonal STAT-1 p84/p91 or STAT-3 antibody was added to the preincubation reaction and incubated for an additional 60 min at 4°C. Protein-DNA complexes were run on a 6% non-denaturing polyacrylamide gel in 0.5x TBE buffer (90 mM Tris, 64.6 mM boric acid, 2.5 mM EDTA); gels were dried and autoradiographs were exposed (Kodak biomax MS film at -70°C).

Statistical analysis. Statistical analysis was performed by unpaired *t*-test. All values are mean ± SEM.

Example 2: 5'-Genomic Sequence of Murine SOCS-3 and Determination of the Transcription Start Site by 5'-RACE and RNase Protection Assay.

Based on the sequence information from the 5' genome walk, a full length 5' product of murine SOCS-3 spanning ~3.8-kb of genomic sequence was generated from ICR Swiss mice genomic DNA by PCR with Advantage®-GC genomic polymerase using the following sense and antisense primers: 5'-GACGTTCCTAAAAGCATGCATGTCACCCAG-3' a (SEQ.ID.NO.:28) nd 5'-GGATCTGCGCGGCGGTGGCTGCAGCTGCTT-3' (SEQ.ID.NO.:29). Cloning of the product into PCR2.1 vector was followed by verification of sense orientation, sequencing, restriction enzyme digestion with *SstI* and *XhoI*, and subcloning of a ~3.7-kb construct into pGL3Basic vector (clone 6). Sequence information was obtained for the whole 3.8 kb. (SEQ. ID. NO.:1).

5'-RACE revealed the existence of an untranslated exon 1 (+1 to +289), separated from exon 2 (starting at +854) by an intron (+290 to +853). Using Rnase protection assay, the main transcription start site was defined and is referred to as +1. The previously determined translation initiation site for murine SOCS-3 (GenBank Accession U88328) (R. Starr *et al.*, Nature 387:917-21 [1997]) was in exon 2 at +944.

Example 3: Effects of Different Cytokines on SOCS-3 Promoter Activity and Gene Expression

Figure 1 shows the stimulatory effect of various cytokines on expression from the SOCS-3 promoter sequence. AtT-20 cells were either untreated, or stimulated with 0.5 x 10^{-9} M LIF, IL-6, or IL-11 for 60 or 120 min. Northern blot analysis showed a SOCS-3-specific signal of uniform transcript size of ~2.8 kb (Fig. 1A).

LIF was the most potent inducer of SOCS-3 mRNA expression. Although IL-6 and IL-11 were less potent stimuli of SOCS-3 gene expression, they each showed a similar pattern of SOCS-3 mRNA induction. (Fig. 1A).

For measurement of SOCS-3 promoter activity, transient transfections of AtT-20 cells

were performed either with pGLBasic alone or with clone 6, a construct containing nucleotides -2,759 to +927 of the 5'-genomic region of murine SOCS-3 linked to the luciferase reporter gene in pGL3Basic vector. (Figure 1B). Relative SOCS-3 promoter activity is indicated by relative light unit values in Figure 1B, calculated from 4 independently performed experiments. Each experiment was performed with n = 3 wells per group. Asterisks indicate in-group significance of untreated (-) vs. treated (+); *, $P<0.05$:**, $P<0.01$. AtT-20 cells transfected with clone 6 showed a significantly higher basal luciferase activity than cells transfected with pGL3Basic alone (4043 ± 443 vs. 1611 ± 398 relative light units [RLU]; $P<0.001$). Stimulation with 0.5 x 10^{-9} M LIF, IL-6, or IL-11 caused no further increase of luciferase activity in control AtT-20 cells transfected with pGL3Basic alone. However, in comparison to untreated cells, AtT-20 cells transfected with clone 6 showed an approximately 10-fold ($P<0.01$) increase in luciferase activity following stimulation with LIF, a 2-fold (not significant) increase following stimulation with IL-6, and a 3-fold ($P<0.05$) stimulation of luciferase activity by IL-11. (Fig. 1B).

Activation by LIF, IL-6 and IL-11, of SOCS-3 promoter activity and gene expression thus is concordant with our finding of a functionally important STAT-1/STAT-3 binding element in the murine SOCS-3 promoter region.

Example 4: Effect of Overexpressed Dominant Negative STAT-3 Mutants or Wild Type SOCS-3 on LIF-induced SOCS-3 Gene Expression and Promoter Activity

Expression from the SOCS-3 promoter is partly dependent both on the expression of STAT-3 and SOCS-3 itself. Figure 2 shows the effect of overexpressed dominant negative STAT-3 mutant or wild type SOCS-3 on LIF-induced SOCS-3 gene expression and promoter activity in AtT-20 cells. AtT-20 cells overexpressing wild type STAT-3 (AtT-20W) showed a 5.4 ± 0.7-fold increase of SOCS-3 mRNA levels after stimulation with 0.5 x 10^{-9} M LIF for 45 min. In comparison, AtT-20 cells overexpressing the dominant negative mutants STAT-3F (AtT-20F) and STAT-3D (AtT-20D) both showed relatively diminished induction of SOCS-3 mRNA after stimulation with LIF: 3.4 ± 0.4 ($p=0.07$) and 2.6 ± 0.1-fold ($p<0.02$), respectively. (Fig. 2A, 2B). Similarly, transient transfection experiments with clone 6 showed stimulation of luciferase activity by LIF (6.9 ± 0.5-fold) in AtT-20W cells,

but only 5.4 ± 0.5- (P=0.09) and 3.4 ± 0.4-fold (P<0.01) stimulation were observed in AtT-20F and AtT-20D cells, respectively. (Fig. 2C). These results, showing that LIF-induced SOCS-3 promoter activity and gene expression is decreased in these dominant negative STAT-3 mutant transfectants, indicate that SOCS-3 promoter activity is at least partly dependent on wild type STAT-3 expression. (Fig. 2A-C).

Overexpression of wild type SOCS-3 in AtT-20 cells abrogated LIF-induced SOCS-3 promoter activity and gene expression. (Fig. 2D-F). Mock-transfected AtT-20 cells (AtT-20M) showed an approximately 5-fold increase of SOCS-3 mRNA levels after 45 min stimulation with 0.5 x 10^{-9} M LIF, while AtT-20 cells overexpressing wild type SOCS-3 (AtT-20S), showed a significant inhibition of LIF-induced SOCS-3 mRNA expression. (Fig. 2D, 2E). Similarly, transient transfection experiments with clone 6 revealed luciferase activity to be stimulated by LIF (9.9 ± 1.3-fold) in AtT-20M cells, while LIF-induced luciferase activity in AtT-20S cells was abrogated and did not differ substantially from luciferase activity in untreated AtT-20S cells. (Fig. 2F). These results indicate a negative autoregulatory feedback of SOCS-3 on its own cytokine-induced gene expression.

LIF-induced SOCS-3 mRNA and luciferase activity were each calculated from 3 independently performed experiments. Each experiment was performed with 3 different clones per group. LIF-induced luciferase activity was normalized to the untreated control for each clone.

Example 5: Functional Analysis of Different SOCS-3 5'Region-luciferase constructs.

Clone 6 is the -2757 to +929 5' genomic region of murine SOCS-3 linked to the luciferase reporter in pGL3Basic vector. 3'-Truncations of clone 6 were: clone 4 (nt. -2759 to +104) and clone 2 (nt. -2759 to -716). 5'-Truncations of clone 6 were: clone 6T1 (nt. -1864 to +927); clone 6T2 (nt -857 to +927); clone 6T3 (nt. -152 to +927); and clone 6T4 (nt. -63 to +927). Analysis of clone 6 sequence with Mat Inspector V2.2 (K. Quandt *et al.*, Nucleic Acids Res. 23: 4878-84 [1995]), revealed potential STAT binding sites containing the consensus binding sequence TT(N)$_5$AA (C.M. Horvath *et al.*, Genes Dev. 9:984-94 [1995]; J.E. Darnell, Jr., Science 277:1630-35 [1997]; S. Becker *et al.*, Nature 394:145-51 [1998]) located at nt. -97 to -89 and nt. -74 to -66, as well as at nt. -347 to -339 and -1403 to -1395. However, only the STAT binding site from nt. -74 to -66 showed the more specific

sequence TTCCAGGAA, indicating a potential binding site for STAT-1 and STAT-3.

Therefore, in subsequent experiments, the focus centered on the STAT binding site at nt. -74 to -66, constituting part of the tandem STAT binding region pair of nt. -97 to -89 and nt. -74 to -66. Using overlap extension PCR, we deleted the complete tandem STAT binding region from nt. -101 to -62 (clone 6D2), or only the 3'-located STAT binding element from nt. -80 to -60 (clone 6D1). In clone 6M1, the 3'-located STAT binding element from nt. -74 to -66 was not deleted, but mutated to ATCGACGAT, thus destroying the specific binding sequence TTCCAGGAA (SEQ.ID.NO.:13). Clone 8 was a minimal -275 to +158 5' genomic region of SOCS-3 linked to the luciferase reporter in pGL3Basic vector. Basal and LIF-induced luciferase activity were assayed after transient transfection of corticotroph AtT-20 cells with the different constructs.

Figure 3 shows relative luciferase activities in transfected AtT-20 cells bearing the different constructs. Relative luciferase activities were calculated in comparison to basal luciferase activity of pGL3Basic alone without LIF treatment, which was defined as 1.0. Basal luciferase activity of clone 2 did not differ from pGL3Basic, and neither clone 2 nor pGL3Basic showed induction of luciferase activity by LIF. However, clones 4 and 6 showed 7- and 4- fold higher basal luciferase activity, respectively, as well as 35-fold higher LIF-stimulated luciferase activity, compared to the pGL3Basic ($P<0.01$). This indicates that the region from nt. +104 to +927 is not involved in SOCS-3 promoter activity.

Increasing 5'-truncations of clone 6 up to nt. -161 caused a gradual increase of basal and LIF-stimulated luciferase activity, with both clone 6T2 ($P<0.01$) and clone 6T3 ($P<0.001$) showing significantly higher basal and LIF-induced luciferase activities than clone 6. Clone 6T3 had the highest basal (17-fold elevation) and LIF-induced (97-fold elevation) luciferase activities, compared to basal pGL3Basic ($p<0.001$). This demonstrates that the region from nt. -2759 to -161 contains apparent negative regulator elements, but is not responsible for basal and LIF-induced SOCS-3 promoter activity.

Further 5'-truncation to nt. -63 in clone 6T4 caused decreases in basal activity and, more markedly, in LIF-inducible promoter activity. Mutated clones 6D1 ($P<0.001$) and 6M1 ($P<0.01$) showed reduced LIF-induced luciferase activity, compared to wild type clone 6. (Fig. 3). Extending the deletion to the entire tandem STAT binding region in clone 6D2,

showed no significant difference in the magnitude of basal vs. LIF-induced luciferase activity in comparison to clone 6D1. These results indicate that the specific STAT-1/STAT-3 binding element at -74 to -66 (TTCCAGGAA; SEQ.ID.NO.:13) mediates the LIF-induced rise in luciferase activity, while the more 5'-located STAT binding element at -97 to -89 (TTACAAGAA; SEQ.ID.NO.:30) does not significantly participate in this signal.

Clone 8 showed basal and LIF-induced luciferase activity comparable to clone 6. (Fig. 3). This further demonstrates the functional importance for SOCS-3 promoter activity of the region containing the STAT-1/STAT-3 binding element.

Example 6: Electromobility Shift Assay

EMSA showed specific binding of nuclear extracts from LIF-induced AtT-20 cells to a double stranded oligonucleotide probe spanning nt. -77 to -57 (STAT oligoprobe), including the STAT-1/STAT-3 binding element from -74 to -66. While nuclear extracts from unstimulated AtT-20 cells did not form specific complexes with the oligoprobe, nuclear extracts from LIF-stimulated AtT-20 cells formed three specific complexes, compatible with STAT-3 homodimers, STAT-1/STAT-3 heterodimers and STAT-1 homodimers (C.M. Horvath *et al.*, Genes Dev. 9:984-94 [1995]; J.E. Darnell, Jr., Science 277:1630-35 [1997]; S. Becker *et al.*, Nature 394:145-51 [1998]). The three complexes disappeared during self-competition with a 100-fold excess of unlabeled double stranded STAT oligonucleotide, whereas the same double stranded oligonucleotide mutated at positions -74, -71, -69, and -66, or a nonspecific double stranded AP-2 oligonucleotide had no effect. Incubation with a specific antibody directed against STAT-1 abolished the two bands representing STAT-1 homodimer and STAT1/STAT3 heterodimer. Similarly, incubation with a specific antibody directed against STAT-3 abolished the two bands representing STAT-3 homodimer and STAT1/STAT3 heterodimer. These results are evidence of specific binding of STAT-1 and STAT-3 to the SOCS-3 promoter region between nt. -74 to -66.

The foregoing examples being illustrative but not an exhaustive description of the embodiments of the present invention, the following claims are presented.

CLAIMS

1. A nucleic acid construct, comprising a murine SOCS-3 promoter having a nucleotide sequence (SEQ. ID. NO:1), or an operative fragment or non-murine homologue thereof, or an operative derivative of any of these.

2. The nucleic acid construct of Claim 1, further comprising said SOCS-3 promoter operatively linked, in a transcriptional unit, to a DNA sequence encoding a preselected protein or to a gene-specific part thereof.

3. The nucleic acid construct of Claim 2, wherein the SOCS-3 promoter is operatively linked to a DNA sequence that encodes a preselected gene-specific antisense RNA.

4. The nucleic acid construct of Claim 2, wherein the DNA sequence encodes a catalytic RNA that specifically hybridizes and cleaves a predetermined functional mRNA.

5. The nucleic acid construct of Claim 2, wherein the preselected protein is a SOCS-3 protein, or a functional fragment thereof.

6. The nucleic acid construct of Claim 2, wherein the SOCS-3 promoter is operatively linked to a DNA sequence that encodes an antisense RNA that specifically hybridizes to a SOCS-3 mRNA.

7. The nucleic acid construct of Claim 4, wherein the predetermined mRNA is a functional SOCS-3 mRNA.

8. The nucleic acid construct of Claim 2, wherein said SOCS-3 promoter is operatively linked to a DNA sequence encoding a reporter protein.

9. The nucleic acid construct of Claim 8, wherein the reporter protein is a fluorescent or light-emitting protein.

10. The nucleic acid construct of Claim 9, wherein the encoded fluorescent or light-emitting protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase, or apoaequorin.

11. The nucleic acid construct of Claim 1, wherein the operative fragment is (SEQ. ID. NO.:2), (SEQ. ID. NO.:3), (SEQ. ID. NO.:4), (SEQ. ID. NO.:5). (SEQ. ID. NO.:6), (SEQ. ID. NO.:7), (SEQ. ID. NO.:8), (SEQ. ID. NO.:9), (SEQ. ID. NO.:10), (SEQ. ID. NO.:11), or (SEQ. ID. NO.:12), or a non-murine homologue, or operative derivative of any of these.

12. The nucleic acid construct of Claim 2, further comprising at least one insulator element flanking said transcriptional unit.

13. The nucleic acid construct of Claim 12, wherein at least one of said insulator element(s) is a chicken β-globin insulator element.

14. A transgenic vertebrate cell containing the nucleic acid construct of Claim 1.

15. The transgenic vertebrate cell of Claim 14, wherein the cell is a pituitary, adrenal, hypothalamic, intestinal, liver, kidney, immune-competent, or bone-forming cell.

16. The transgenic vertebrate cell of Claim 14, wherein the cell is a hepatocyte, lymphocyte, chondrocyte, neuron, intestinal epithelial, corticotroph, somatotroph, lactotroph, or gonadotroph cell.

17. The transgenic vertebrate cell of Claim 14, wherein the cell is a cell derived from a pituitary tumor, adrenal tumor, hypothalamic tumor, or liver tumor.

18. The transgenic vertebrate cell of Claim 14, grown in the presence of an inhibitor of DNA methylation.

19. A transgenic non-human vertebrate comprising the cell of Claim 14.

20. The transgenic non-human vertebrate of Claim 19, wherein said vertebrate is a non-human mammal or a bird.

21. A kit for genetically modifying a vertebrate cell, containing a polynucleotide comprising a murine SOCS-3 promoter sequence having SEQ. ID. NO.:1, or an operative fragment or non-murine homologue thereof, or an operative derivative of any of these.

22. The kit of Claim 21, further comprising said SOCS-3 promoter operatively linked, in a transcriptional unit, to a DNA sequence encoding a preselected protein or to a gene-specific part thereof.

23. The kit of Claim 22, wherein the SOCS-3 promoter is operatively linked to a DNA sequence that encodes a preselected gene-specific antisense RNA.

24. The kit of Claim 22, wherein the SOCS-3 promoter is operatively linked to a DNA sequence that encodes a catalytic RNA that specifically hybridizes and cleaves a predetermined functional mRNA.

25. The kit of Claim 22, wherein the preselected protein is a SOCS-3 protein, or a functional fragment thereof.

26. The kit of Claim 22, wherein the DNA sequence encodes a SOCS-3 protein or fragment thereof; and the DNA sequence is adjoined to the SOCS-3 promoter in an antisense orientation.

27. The kit of Claim 24, wherein the predetermined mRNA is a functional SOCS-3 mRNA.

28. The kit of Claim 22, wherein said SOCS-3 promoter is operatively linked to a DNA sequence encoding a reporter protein.

29. The kit of Claim 28, wherein the encoded reporter protein is a fluorescent or light-emitting protein.

30. The kit of Claim 29, wherein the encoded fluorescent or light-emitting protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase, or apoaequorin.

31. The kit of Claim 22, wherein said polynucleotide further comprises at least one insulator element flanking said transcriptional unit.

32. The kit of Claim 31, wherein at least one of said insulator element(s) is a chicken β-

globin insulator element.

33. The kit of Claim 21, further comprising a transfecting agent.

34. The kit of Claim 33, wherein the transfecting agent is a liposome, viral vector, transferrin-polylysine enhanced viral vector, retroviral vector, lentiviral vector, or uptake enhancing DNA segment, or a mixture of any of these.

35. The kit of Claim 33, wherein the transfecting agent comprises a retroviral vector, adenoviral vector, transferrin-polylysine enhanced adenoviral vector, human immunodeficiency virus vector, lentiviral vector, Moloney murine leukemia virus-derived vector, mumps vector, a DNA segment that facilitates polynucleotide uptake by and release into the cytoplasm of vertebrate cells, or comprises an operative fragment of or mixture of any of these.

36. The kit of Claim 33, wherein the transfecting agent comprises an adenovirus vector having endosomal lytic activity, and the polynucleotide is operatively linked to the vector.

37. The kit of Claim 33, wherein the transfecting agent comprises a lipid transfecting agent.

38. A method of treating a growth retardation disorder in a human subject, comprising:

genetically modifying a GH-responsive or gp-130-responsive cell of a human subject having a growth retardation disorder with a nucleic acid construct comprising a SOCS-3 promoter sequence or operative fragment thereof, operatively linked, in a transcriptional unit, to a DNA sequence encoding an RNA that specifically hybridizes to a functional SOCS-3 mRNA; and

causing the cell, in vivo, to transcribe from said transcriptional unit, in response to a growth-inducing cytokine, an RNA transcript that specifically hybridizes to a functional SOCS-3 mRNA, preventing translation therefrom, whereby the amount of SOCS-3 protein produced in the cell is reduced and one or more symptoms of the growth retardation disorder is improved.

39. The method of Claim 38, wherein the cell is a pituitary, adrenal, hypothalamic, liver, immune-competent, or bone-forming cell.

40. The method of Claim 38, wherein the cell is a hepatocyte, lymphocyte, chondrocyte,

corticotroph, somatotroph, lactotroph, or gonadotroph cell.

41. The method of Claim 38, wherein the cell is derived from a pituitary tumor, adrenal tumor, hypothalamic tumor, liver, or bone tumor.

42. The method of Claim 38, wherein the SOCS-3 promoter sequence is a murine SOCS-3 promoter having a nucleotide sequence (SEQ. ID. NO:1), or an operative fragment or non-murine homologue thereof, or an operative derivative of any of these.

43. The method of Claim 38, wherein the DNA sequence encodes a catalytic RNA that specifically hybridizes to and cleaves a functional SOCS-3 mRNA.

44. The method of Claim 38, wherein said transcriptional unit further comprises a DNA sequence encoding a reporter protein.

45. The method of Claim 44, wherein the encoded reporter protein is a fluorescent or light-emitting protein.

46. The method of Claim 45, wherein the encoded fluorescent or light-emitting protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase, or apoaequorin.

47. The method of Claim 38, further comprising at least one insulator element flanking said transcriptional unit.

48. The method of Claim 47, wherein at least one of said insulator element(s) is a chicken β-globin insulator element.

49. The method of Claim 38, wherein the growth-inducing cytokine is human growth hormone or a growth-inducing cytokine of the gp130 signaling subunit cytokine family.

50. The method of Claim 38, further comprising obtaining the GH-responsive or gp-130-responsive cell from a tissue of the human subject; genetically modifying the cell in vitro; and re-implanting the genetically modified cell into the tissue of the human subject.

51. A method of treating a growth acceleration disorder in a human subject, comprising:

genetically modifying a GH-responsive or gp-130-responsive cell of a human subject having a growth acceleration disorder with a nucleic acid construct comprising a SOCS-3 promoter sequence or operative fragment thereof, operatively linked, in a transcriptional unit, to a DNA sequence encoding a SOCS-3 protein or a functional fragment thereof; and

causing the cell, in vivo, to transcribe from said transcriptional unit, in response to a growth-inducing cytokine, a functional SOCS-3 mRNA , whereby the amount of SOCS-3 protein produced in said cell is enhanced, and one or more symptoms of the growth acceleration disorder is improved.

52. The method of Claim 51, wherein the cell is a pituitary, adrenal, hypothalamic, liver, immune-competent, or bone-forming cell.

53. The method of Claim 51, wherein the cell is a hepatocyte, lymphocyte, chondrocyte, corticotroph, somatotroph, lactotroph, or gonadotroph cell.

54. The method of Claim 51, wherein the cell is a cell derived from a pituitary tumor, adrenal tumor, hypothalamic tumor, liver, or bone tumor.

55. The method of Claim 51, wherein the SOCS-3 promoter sequence is a murine SOCS-3 promoter having a nucleotide sequence (SEQ. ID. NO:1), or an operative fragment or non-murine homologue thereof, or an operative derivative of any of these.

56. The method of Claim 51, wherein said transcriptional unit further comprises a DNA sequence encoding a reporter protein.

57. The method of Claim 56, wherein the encoded reporter protein is a fluorescent or light-emitting protein.

58. The method of Claim 57, wherein the encoded fluorescent or light-emitting protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase, or apoaequorin.

59. The method of Claim 51, further comprising at least one insulator element flanking said transcriptional unit.

60. The method of Claim 59, wherein at least one of said insulator element(s) is a chicken β-globin insulator element.

61. The method of Claim 51, wherein the growth-inducing cytokine is human growth hormone or a cytokine of the gp130 signaling subunit cytokine family.

62. The method of Claim 51, further comprising obtaining the GH-responsive or gp-130-responsive cell from a tissue of the human subject; genetically modifying the cell in vitro; and re-implanting the genetically modified cell into the tissue of the human subject.

63. A method of treating an autoimmune disease, immune disease, or an inflammatory condition in a human subject, comprising:

genetically modifying a gp-130-responsive cell of a human subject having an autoimmune disease, immune disease, or inflammatory condition with a nucleic acid construct comprising a SOCS-3 promoter sequence, or fragment thereof, operatively linked, in a transcriptional unit, to a DNA sequence encoding a SOCS-3 protein or a functional fragment thereof; and

causing the cell, in vivo, to transcribe from said transcriptional unit, in response to a a pro-inflammatory cytokine of the gp130 signaling subunit cytokine family, a functional SOCS-3 mRNA, whereby the amount of SOCS-3 protein produced in said cell is enhanced, whereby the amount of SOCS-3 protein produced in the cell is enhanced, and one or more symptoms of the autoimmune disease, immune disease, or inflammatory condition is improved.

64. The method of Claim 63, wherein the cell is a pituitary, adrenal, hypothalamic, intestinal, kidney, liver, immune-competent, or bone-forming cell.

65. The method of Claim 63, wherein the cell is a hepatocyte, lymphocyte, chondrocyte, neuron, intestinal epithelial, corticotroph, somatotroph, lactotroph, or gonadotroph cell.

66. The method of Claim 63, wherein the cell is a cell derived from a pituitary tumor, adrenal tumor, hypothalamic tumor, or liver tumor.

67. The method of Claim 63, wherein the SOCS-3 promoter sequence is a murine SOCS-3 promoter having a nucleotide sequence (SEQ. ID. NO:1), or an operative fragment or non-murine homologue thereof, or an operative derivative of any of these.

68. The method of Claim 63, wherein said transcriptional unit further comprises a DNA sequence encoding a reporter protein.

69. The method of Claim 68, wherein the encoded reporter protein is a fluorescent or light-emitting protein.

70. The method of Claim 69, wherein the encoded fluorescent or light-emitting protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase, or apoaequorin.

71. The method of Claim 63, further comprising at least one insulator element flanking said transcriptional unit.

72. The method of Claim 71, wherein at least one of said insulator element(s) is a chicken β-globin insulator element.

73. The method of Claim 63, wherein the pro-inflammatory cytokine is IL-6 or LIF.

74. The method of Claim 63, wherein the autoimmune disease, immune disease, or inflammatory condition is Crohn's disease, ulcerative colitis, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, Grave's disease, an allergic or anaphylactic reaction, or a neuroendocrinological response to stress.

75. The method of Claim 63, further comprising obtaining the gp130-responsive cell from a tissue of the human subject; genetically modifying the cell in vitro; and re-implanting the genetically modified cell into the tissue of the human subject.

76. A method of treating an autoimmune disease, immune disease, or an inflammatory condition in a human subject, comprising:

genetically modifying a gp-130-responsive cell of a human subject having an autoimmune

disease, immune disease, or inflammatory condition with a nucleic acid construct comprising a SOCS-3 promoter sequence, or fragment thereof, operatively linked, in a transcriptional unit, to a DNA sequence encoding a functional anti-inflammatory cytokine of the gp130 subunit signaling cytokine family linked to a functional secretory signal; and

causing the cell, in vivo, to transcribe from said transcriptional unit, in response to a a pro-inflammatory cytokine of the gp130 signaling subunit cytokine family, a functional mRNA transcript, whereby the anti-inflammatory cytokine is produced and secreted in response to the pro-inflammatory cytokine, and one or more symptoms of the autoimmune disease, immune disease, or inflammatory condition is improved.

77. The method of Claim 76, wherein the cell is a pituitary, adrenal, hypothalamic, intestinal, kidney, liver, immune-competent, or bone-forming cell.

78. The method of Claim 76, wherein the cell is a hepatocyte, lymphocyte, chondrocyte, neuron, intestinal epithelial, corticotroph, somatotroph, lactotroph, or gonadotroph cell.

79. The method of Claim 76, wherein the cell is a cell derived from a pituitary tumor, adrenal tumor, hypothalamic tumor, or liver tumor.

80. The method of Claim 76, wherein the SOCS-3 promoter sequence is a murine SOCS-3 promoter having a nucleotide sequence (SEQ. ID. NO:1), or an operative fragment or non-murine homologue thereof, or an operative derivative of any of these.

81. The method of Claim 76, wherein said transcriptional unit further comprises a DNA sequence encoding a reporter protein.

82. The method of Claim 81, wherein the encoded reporter protein is a fluorescent or light-emitting protein.

83. The method of Claim 82, wherein the encoded fluorescent or light-emitting protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase, or apoaequorin.

84. The method of Claim 76, further comprising at least one insulator element flanking

said transcriptional unit.

85. The method of Claim 84, wherein at least one of said insulator element(s) is a chicken β-globin insulator element.

86. The method of Claim 76, wherein the pro-inflammatory cytokine is IL-6 or LIF.

87. The method of Claim 76, wherein the anti-inflammatory cytokine is IL-11.

88. The method of Claim 76, wherein the autoimmune disease, immune disease, or inflammatory condition is Crohn's disease, ulcerative colitis, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, Grave's disease, or a neuroendocrinological response to stress.

89. The method of Claim 76, further comprising obtaining the gp130-responsive cell from a tissue of the human subject; genetically modifying the cell in vitro; and re-implanting the genetically modified cell into the tissue of the human subject.

90. A method of treating a growth retardation disorder in a human subject, comprising:

obtaining a GH-responsive or gp-130-responsive cell from a tissue of a human subject having a growth retardation disorder;

genetically modifying said cell, in vitro, with a nucleic acid construct comprising a a murine SOCS-3 promoter having a nucleotide sequence (SEQ. ID. NO:1), or an operative fragment or non-murine homologue thereof, or an operative derivative of any of these, operatively linked, in a transcriptional unit, to a DNA sequence encoding a SOCS-3 protein or to a gene-specific part thereof, such that transcription of the DNA sequence, from said transcriptional unit, in response to a growth-inducing cytokine, results in an RNA transcript that specifically hybridizes to a functional SOCS-3 mRNA, whereby the amount of SOCS-3 protein produced in the cell is reduced;

re-implanting said cell within the tissue of said human subject, whereby the amount of SOCS-3 protein produced, in vivo, in response to the growth-inducing cytokine is reduced, and one or more symptoms of the growth retardation disorder is improved.

91. The method of Claim 90, wherein the cell is a pituitary, adrenal, hypothalamic, liver, immune-competent, or bone-forming cell.

92. The method of Claim 90, wherein the cell is a hepatocyte, lymphocyte, chondrocyte, corticotroph, somatotroph, lactotroph, or gonadotroph cell.

93. The method of Claim 90, wherein the cell is a cell derived from a pituitary tumor, adrenal tumor, hypothalamic tumor, liver tumor, or bone tumor.

94. The method of Claim 90, wherein the DNA sequence encodes a catalytic RNA that specifically hybridizes and cleaves a functional SOCS-3 mRNA.

95. The method of Claim 90, wherein said transcriptional unit further comprises a DNA sequence encoding a reporter protein.

96. The method of Claim 95, wherein the encoded reporter protein is a fluorescent or light-emitting protein.

97. The method of Claim 96, wherein the encoded fluorescent or light-emitting protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase, or apoaequorin.

98. The method of Claim 90, further comprising at least one insulator element flanking said transcriptional unit.

99. The method of Claim 98, wherein at least one of said insulator element(s) is a chicken β-globin insulator element.

100. The method of Claim 90, wherein the growth-inducing cytokine is human growth hormone or a cytokine of the gp130 signaling subunit cytokine family.

101. A method of treating a growth acceleration disorder in a human subject, comprising:

obtaining a GH-responsive or gp-130-responsive cell from a tissue of a human subject having a growth acceleration disorder,

genetically modifying said cell, in vitro, with a nucleic acid construct comprising a a murine SOCS-3 promoter having a nucleotide sequence (SEQ. ID. NO:1), or an operative fragment or non-murine homologue thereof, or an operative derivative of any of these, operatively linked, in a

transcriptional unit, to a DNA sequence encoding a SOCS-3 protein or operative fragment thereof; and

re-implanting said cell within the tissue of said human subject, whereby the amount of SOCS-3 protein produced in said cell, in vivo, in response to a growth-inducing cytokine is enhanced, and one or more symptoms of the growth acceleration disorder is improved.

102. The method of Claim 101, wherein the cell is a pituitary, adrenal, hypothalamic, liver, immune-competent, or bone-forming cell.

103. The method of Claim 101, wherein the cell is a hepatocyte, lymphocyte, chondrocyte, corticotroph, somatotroph, lactotroph, or gonadotroph cell.

104. The method of Claim 101, wherein the cell is a cell derived from a pituitary tumor, adrenal tumor, hypothalamic tumor, liver tumor, or bone tumor.

105. The method of Claim 101, wherein said transcriptional unit further comprises a DNA sequence encoding a reporter protein.

106. The method of Claim 105, wherein the reporter protein is a fluorescent or light-emitting protein.

107. The method of Claim 106, wherein the fluorescent or light-emitting protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase, or apoaequorin.

108. The method of Claim 101, further comprising at least one insulator element flanking said transcriptional unit.

109. The method of Claim 108, wherein at least one of said insulator element(s) is a chicken β-globin insulator element.

110. The method of Claim 101, wherein the growth-inducing cytokine is human growth hormone or a cytokine of the gp130 signaling subunit family.

111. A method of treating an autoimmune disease, immune disease, or inflammatory condition in a human subject, comprising:

obtaining a gp130 responsive cell from a tissue of a human subject having an autoimmune disease, immune disease, or inflammatory condition;

transfecting said cell with a nucleic acid construct comprising a murine SOCS-3 promoter having a nucleotide sequence (SEQ. ID. NO:1), or an operative fragment or non-murine homologue thereof, or an operative derivative of any of these, operatively linked, in a transcriptional unit, to a DNA sequence encoding a SOCS-3 protein or a functional fragment thereof; and

re-implanting said cell within the tissue of said human subject, whereby the amount of SOCS-3 protein produced in said cell in response to the pro-inflammatory cytokine is enhanced, and one or more of the symptoms of the autoimmune disease, immune disease, or inflammatory condition is improved.

112. The method of Claim 111, wherein the cell is a pituitary, adrenal, hypothalamic, intestinal, kidney, immune-competent, or liver cell.

113. The method of Claim 111, wherein the cell is a hepatocyte, lymphocyte, corticotroph, somatotroph, lactotroph, or gonadotroph cell.

114. The method of Claim 111, wherein the cell is a cell derived from a pituitary tumor, adrenal tumor, hypothalamic tumor, or liver tumor.

115. The method of Claim 111, wherein said transcriptional unit further comprises a DNA sequence encoding a reporter protein.

116. The method of Claim 115, wherein the encoded reporter protein is a fluorescent or light-emitting protein.

117. The method of Claim 116, wherein the fluorescent or light-emitting protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase, or apoaequorin.

118. The method of Claim 111, further comprising at least one insulator element flanking said transcriptional unit.

119. The method of Claim 118, wherein at least one of said insulator element(s) is a chicken β-globin insulator element.

120. The method of Claim 111, wherein the pro-inflammatory cytokine is IL-6 or LIF.

121. The method of Claim 47, wherein the autoimmune disease, immune disease, or inflammatory condition is Crohn's disease, ulcerative colitis, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, Grave's disease, an allergic or anaphylactic reaction, or a neuroendocrinological response to stress.

122. A method of treating an autoimmune disease, immune disease, or inflammatory condition in a human subject, comprising:

obtaining a gp130-responsive cell from a tissue of a human subject having an autoimmune disease, immune disease, or inflammatory condition;

transfecting said cell with a nucleic acid construct comprising a murine SOCS-3 promoter having a nucleotide sequence (SEQ. ID. NO:1), or an operative fragment or non-murine homologue thereof, or an operative derivative of any of these, operatively linked, in a transcriptional unit, to a DNA sequence encoding a functional anti-inflammatory cytokine of the gp130 subunit signaling cytokine family linked to a functional secretory signal; and

re-implanting said cell within the tissue of said human subject, whereby the anti-inflammatory cytokine is produced and secreted, in vivo, in response to a pro-inflammatory cytokine of the gp130 subunit signaling cytokine family, and one or more symptoms of the autoimmune disease, immune disease, or inflammatory condition is improved.

123. The method of Claim 122, wherein the cell is a pituitary, adrenal, hypothalamic, intestinal, kidney, liver, immune-competent, or bone-forming cell.

124. The method of Claim 122, wherein the cell is a hepatocyte, lymphocyte, chondrocyte, intestinal epithelial, corticotroph, somatotroph, lactotroph, or gonadotroph cell.

125. The method of Claim 122, wherein the cell is a cell derived from a pituitary tumor, adrenal tumor, hypothalamic tumor, or liver tumor.

126. The method of Claim 122, wherein said transcriptional unit further comprises a DNA

sequence encoding a reporter protein.

127. The method of Claim 126, wherein the encoded reporter protein is a fluorescent or light-emitting protein.

128. The method of Claim 127, wherein the fluorescent or light-emitting protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase, or apoaequorin.

129. The method of Claim 122, further comprising at least one insulator element flanking said transcriptional unit.

130. The method of Claim 129, wherein at least one of said insulator element(s) is a chicken β-globin insulator element.

131. The method of Claim 122, wherein the anti-inflammatory cytokine is IL-11.

132. The method of Claim 122, wherein the pro-inflammatory cytokine is IL-6 or LIF.

133. The method of Claim 122, wherein the autoimmune disease, immune disease, or inflammatory condition is Crohn's disease, ulcerative colitis, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, Grave's disease, an allergic or anaphylactic reaction, or a neuroendocrinological response to stress.

Fig. 1A

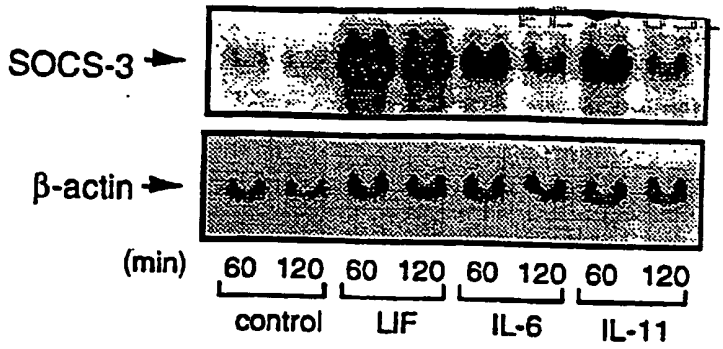


Fig. 1B

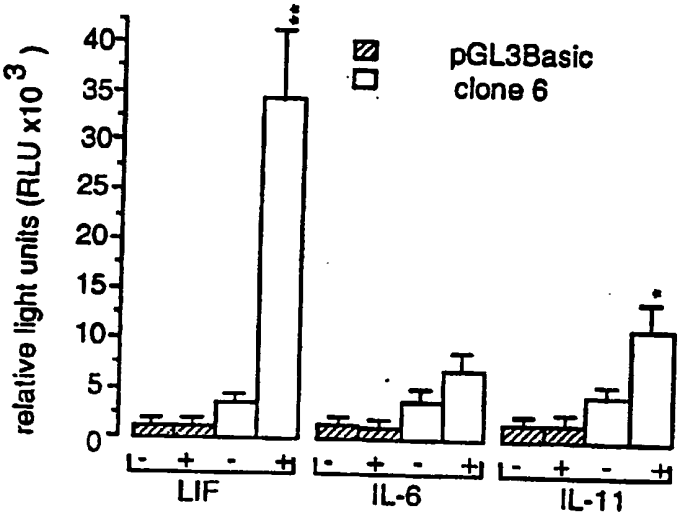


Fig. 2A

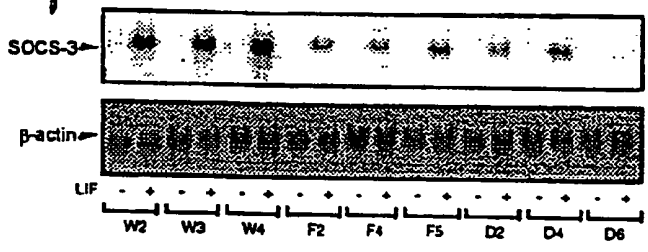


Fig. 2D

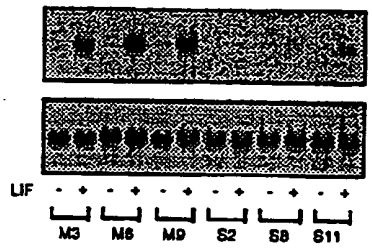
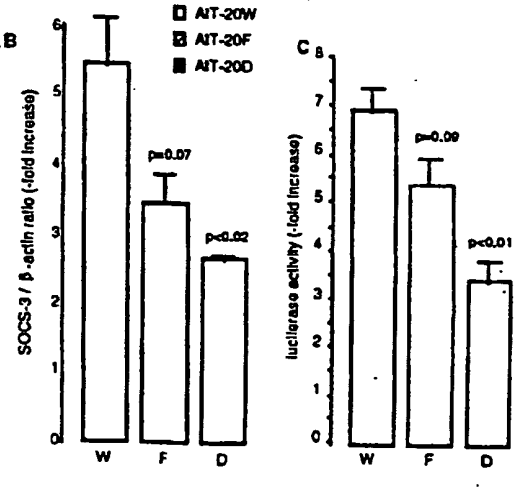


Fig. 2B



C

Fig. 2E

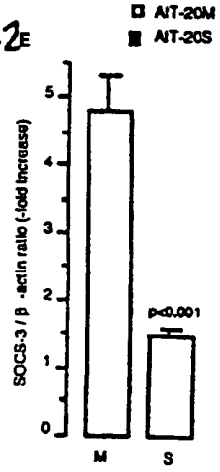


Fig. 2F

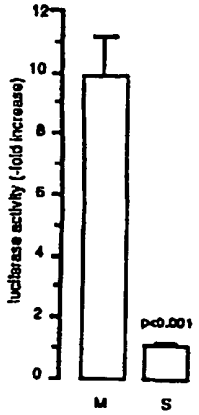
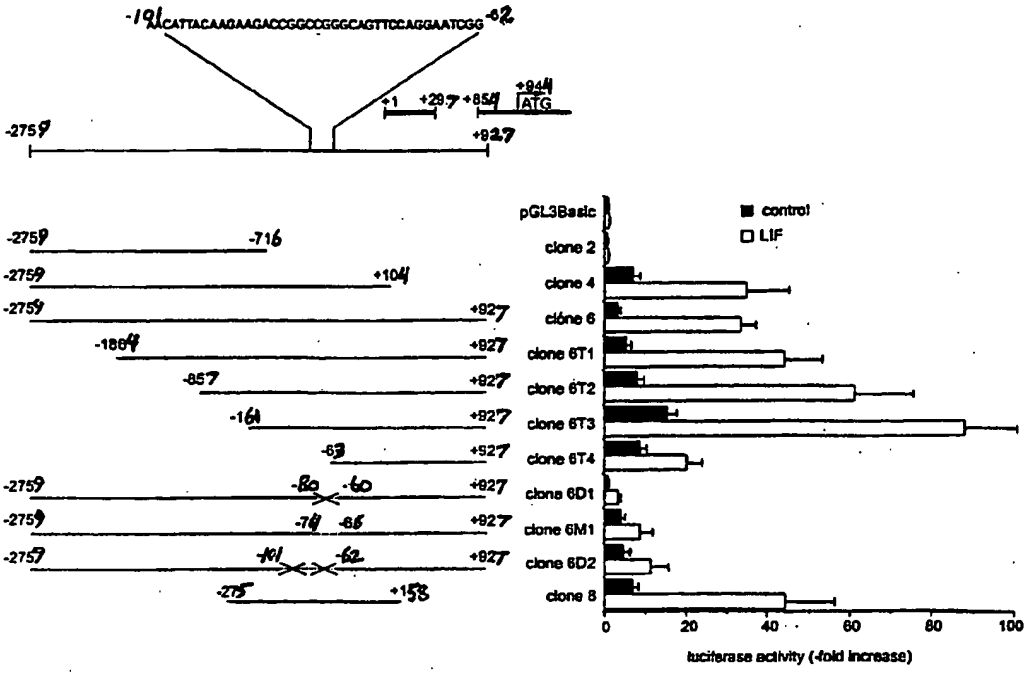


Figure 3


-191
CATTACAAGAAGACCGGCCGGGCAGTTCCAGGAATCGG
-62
+1
+297
+859
+944
ATG
-2759
+927
-716
+104
-1864
-857
-161
-63
-80
-60
-74
-66
-101
-62
-275
+158
pGL3Basic
clone 2
clone 4
clone 6
clone 6T1
clone 6T2
clone 6T3
clone 6T4
clone 6D1
clone 6M1
clone 6D2
clone 8
control
LIF
0
20
40
60
80
100
luciferase activity (-fold increase)

SEQUENCE LISTING

<110> Cedars-Sinai Medical Center (Applicant)

<120> SUPPRESSOR OF CYTOKINE SIGNALING
(SOCS)-3 PROMOTER AND METHODS FOR ITS USE IN GENETIC THERAPY
IN HUMANS

<130> CEDAR-045010

<140> TO BE ASSIGNED
<141> 2000-06-06

<150> 09/327,138
<151> 1999-06-07

<160> 30

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 3940
<212> DNA
<213> Mus musculus

<220>
<221> promoter
<222> (-2907)...(1033)

<221> promoter
<222> (0)...(0)

<221> promoter
<222> (0)...(0)

<221> promoter
<222> (0)...(0)

<221> promoter
<222> (0)...(0)

<221> promoter
<222> (0)...(0)

<400> 1
gacgttccta aaagcatgca tgtcacccag cttacccacc catctcaggc cacagcagcc 60
tgagagagcg gaagaacacc tgctggtcct gtcccacctc tcctcttcaa acagccccac 120
atcctccagt tttgctctgg gtggagctcc ctgctggccc tgcagaggga aggctctcct 180
aagcatcatc tatcagaacg tcttcaaaaa aaaaaaaaaa aaaaaaaaag cctctccagc 240

caggctagct ctaacaccat ttcttccccc tcccctctct caaattcact tatcttttt 300
ttttttttt tttttggatt tttgagacag ggtttctctg tatagccctg gttgtcctgg 360
aactcacttt gtacaccagc ctggcctcga actcagagat ccacctgcct ctgcctcctg 420
agtgctggga ttaaaggcgt gcgccaccac gcccggctaa attcacttat ctatttaatg 480
tatatagggt ataggctgcc cttgaactca caaagatctg cttgctttgc ttctggaata 540
ctaaaggtgt gtgctaccat cacagggacc aagatttatt ttaattctgt atatgtgtgt 600
gtgtgtgtgt gtgtatgggg ggtgcacatg agtacagatt cccttggagg cctggggtgg 660
cttaggactg gggttacaac agttgtgacc catcctacat aggtcctggc accaacaccc 720
ccccccccc cccccgtctt ccagaagtgc agcaggtgtt cttaactgct gagccagcaa 780
tccagcccct gacttccctc tcttacttaa gaagctatca cagtgtctca ctgggtcaca 840
atcatgacta gtccttgctc atggcccaca gcctcttccc cactgtgggt tttgccccgc 900
agctctgccg ccccagcgct gcacccgagg cctgacagag ccaggcacga agtcagggtt 960
tgtggaatgg atgaatgaac ttgactcgtg gcagagcatt gtaatttaca aagcactttc 1020
ccatccatta actccagggc tatttcctaa gagtcctccc tgtcctccac tgccctcggc 1080
tcagaggcat acggtcaagg cagtggctgg ggaacactcc ctgaatgaga tcaaggaggg 1140
cttgttcaca gagaaaggga gaatccattt ggggagcctg agagtgactc gaaggcaagg 1200
actgggcctc acctgtggga tctccatctg tgagcatccg ctcatcagac cagtgtgaga 1260
tattttaaat aaggccccta agcctcttga ctactggaat tgccaggggc gggggacaga 1320
tgggcaccca tccctattta acagataaca agactgagtc cagagaggca gtgcacctgc 1380
cctggtctct cttagttcct cagcatcagt ggagcagatt ggacacagtg ggccagaagg 1440
gaagcaggca gccctccctc ctagcccaag ctactctgtg tagtcagttt gccctcctac 1500
tggtgttaca agaagcctgt ggtatccaag agggcaggtc agaaagccca ctgagagcag 1560
acactgtgtg tcacttagct ggttctcagg tggctgccac ttcctgctgc ctgttgcaaa 1620
actcgacact aggcctttat agatactcac gtgaccagga gtaaacaacc tttcacctca 1680
atcacctgct cttatcaata ctccctctcc accccaccat cgggaaagtt cagacacctt 1740
aaaacgtaga ggcaagagag ggtccattct gacacctcag cgactttcag gcagtggctg 1800
aacccgttac aacgctctgt ggacagtcct cctagtcgac attccttctc aggtttgacc 1860
ctgtcctggg aagtgaggct tctctctctg ggttccccac tcctgttctt gaataaggag 1920
ccccacaacc tcttattctc tctatacaga gcctgggaaa cagcaaaact cggctcgcct 1980
acaagactcc agcgcgccct ctggtggact cgggggacga gcatgggatg agggtttctt 2040
tcctctagct cccccaccgc gccgagagta cctgggcgga cccacagttc gccacgcagg 2100
ttgggaggcc cagatgagtg ataaggtagt agttagctgc tcctcccacc ccactcccca 2160
aaggacatca gcacccacgt ctgtcaccga agaaccaggc aatgggcgga tgagctgagg 2220
ccaggtagct gcttctaagt cagtgtctcc tccacttctg gatctcacag cttcatcttt 2280
tggacctgtc tacaggtaaa tgtcgcgcat ccccctcctc cacttcctag gtccccagtg 2340
ggctggtggc tgaatggtcc tacgtccctt ttggttggca cgggatgctt ggaactgtac 2400
atgaggacct cggggtggcc tgggtgcaga gggaggggag cgtccccgcg gggatcaaaa 2460
gaaagggaag gggtgccagg agggagcctc tcccggctgg cctcctagaa ctgcccgcgc 2520
gctcccatcg cgacgccccc gcctctgcca gaaaccagcc ttcttagaag ggaggggggg 2580
gaaagtgtga atgagaagtt gggggcggag cgcgcggggg aggggccgct gccaggaacg 2640
ctcggccaag gctggcgccg cgcccgccgg tcgggcagcc tcgcgccgcg ctttgtctcc 2700
ctctcggtga gtctcggcgg gtcctggagg ccccagctcc aagcccgccc tccgcagccc 2760
ctccctcgcc ctccgcgcac agcctttcag tgcagagtag tgactaaaca ttacaagaag 2820
accggccggg cagttccagg aatcgggggg cggggcgtac tggccgggta aatacccgcg 2880
cgcgcggcct ccgaggcggc tctaactctg actctacact cgcccgctcc tacgaccgct 2940
gtctctccgg gctcccggac gcccccttcc cggcccagct ctccgtcgag gtccctcgcc 3000
caggtccttt gcctgattcg cccaggagtg cgcctcatcg gcccggggag cagcgaagcc 3060
agaggggcg cacgcacggg gagccccttt gtagacttca cggctgccaa catctgggcg 3120
cagcgcgagc cactgctggg cgccgcctcg cctcggggac cataggaggc gcagccccaa 3180
ggccggagat ttcgcttcgg gactaggtag gaaggagggg cgcggtgtgg ggaagggtgg 3240
gggcatcggt ccagctcggg agcttttccc ggtttctcct ccccttcccg ggtcattccc 3300
ggtagggagg ggacgaggca gggggcagag cggatgagaa ccgaagatcc ctgattcccg 3360
tcatactcag actggggccc tcgggtttct cctgtccccc ctctcacata tctcgggttt 3420
ggcacccccc ttttttcgcc ctcgccactg aggacaccgg actgagaggc gccctgagcg 3480
tccctagggc tcttgtgtct ctccccatcc tggccgcgct cctggagacc caacttccac 3540

gcgcgagttt tctctgggcg tcctcctagg gcgggcaggg gaagagactg tctggggttg 3600
gccggcagtg accgaggaca gtcgagttcc gcgaggtggc tgggcctgag acacggtcta 3660
aagcggggca aaggggtgcc ccgggcgcta ggcggaggct ggagggccgg gcacgctgga 3720
gggttccggg cactcacgcg cctcacgctt tgctctctgc agctccccgg gatgcggtag 3780
cggccgctgt gcggaggccg cgaagcagct gcagccaccg ccgcgcagat ccacgctggc 3840
tccgtgcgcc atggtcaccc acagcaagtt tcccgccgcc gggatgagcc gccccctgga 3900
caccagcctg cgcctcaaga ccttcagctc caaaagcgag 3940

<210> 2
<211> 2863
<212> DNA
<213> Mus musculus

<220>
<221> promoter
<222> (-2759)...(104)

<400> 2
ccctgctggc cctgcagagg gaaggctctc ctaagcatca tctatcagaa cgtcttcaaa 60
aaaaaaaaaa aaaaaaaaaa agcctctcca gccaggctag ctctaacacc atttcttccc 120
cttcccctct ctcaaattca cttatctttt ttttttttt ttttttgga tttttgagac 180
agggtttctc tgtatagccc tggttgtcct ggaactcact ttgtacacca gcctggcctc 240
gaactcagag atccacctgc ctctgcctcc tgagtgctgg gattaaaggc gtgcgccacc 300
acgcccggct aaattcactt atctatttaa tgtatatagg gtataggctg cccttgaact 360
cacaaagatc tgcttgcttt gcttctggaa tactaaaggt gtgtgctacc atcacaggga 420
ccaagattta ttttaattct gtatatgtgt gtgtgtgtgt gtgtgtatgg ggggtgcaca 480
tgagtacaga ttcccttgga ggcctggggt ggcttaggac tggggttaca acagttgtga 540
cccatcctac ataggtcctg gcaccaacac ccccccccc ccccccgtc ttccagaagt 600
gcagcaggtg ttcttaactg ctgagccagc aatccagccc ctgacttccc tctcttactt 660
aagaagctat cacagtgtct cactgggtca caatcatgac tagtccttgc tcatggccca 720
cagcctcttc cccactgtgg gttttgcccc gcagctctgc cgccccagcg ctgcacccga 780
ggcctgacag agccaggcac gaagtcaggg tttgtggaat ggatgaatga acttgactcg 840
tggcagagca ttgtaattta caaagcactt tcccatccat taactccagg gctatttcct 900
aagagtcctc cctgtcctcc actgccctcg gctcagaggc atacggtcaa ggcagtggct 960
ggggaacact ccctgaatga gatcaaggag ggcttgttca cagagaaagg gagaatccat 1020
ttggggagcc tgagagtgac tcgaaggcaa ggactgggcc tcacctgtgg gatctccatc 1080
tgtgagcatc cgctcatcag accagtgtga gatattttaa ataaggcccc taagcctctt 1140
gactactgga attgccaggg gcgggggaca gatgggcacc catccctatt taacagataa 1200
caagactgag tccagagagg cagtgcacct gccctggtct ctcttagttc ctcagcatca 1260
gtggagcaga ttggacacag tgggccagaa gggaagcagg cagccctccc tcctagccca 1320
agctactctg tgtagtcagt ttgccctcct actggtgtta caagaagcct gtggtatcca 1380
agagggcagg tcagaaagcc cactgagagc agacactgtg tgtcacttag ctggttctca 1440
ggtggctgcc acttcctgct gcctgttgca aaactcgaca ctaggccttt atagatactc 1500
acgtgaccag gagtaaacaa cctttcacct caatcacctg ctcttatcaa tactccctct 1560
ccacccccacc atcgggaaag ttcagacacc ttaaaacgta gaggcaagag agggtccatt 1620
ctgacacctc agcgactttc aggcagtggc tgaacccgtt acaacgctct gtggacagtc 1680
ctcctagtcg acattccttc tcaggtttga ccctgtcctg ggaagtgagg cttctctctc 1740
tgggttcccc actcctgttc ttgaataagg agccccacaa cctcttattc tctctataca 1800
gagcctggga aacagcaaaa ctcggctcgc ctacaagact ccagcgcgcc ctctggtgga 1860
ctcgggggac gagcatggga tgagggtttc tttcctctag ctcccccacc gcgccgagag 1920
tacctgggcg gacccacagt tcgccacgca ggttgggagg cccagatgag tgataaggta 1980
gtagttagct gctcctccca ccccactccc caaaggacat cagcacccac gtctgtcacc 2040
gaagaaccag gcaatgggcg gatgagctga ggccaggtag ctgcttctaa gtcagtgtct 2100
cctccacttc tggatctcac agcttcatct tttggacctg tctacaggta aatgtcgcgc 2160
atccccctcc tccacttcct aggtccccag tgggctggtg gctgaatggt cctacgtccc 2220

ttttggttgg cacgggatgc ttggaactgt acatgaggac ctcggggtgg cctgggtgca 2280
gagggagggg agcgtccccg cggggatcaa aagaaaggga aggggtgcca ggagggagcc 2340
tctcccggct ggcctcctag aactgcccgc gcgctcccat cgcgacgccc ccgcctctgc 2400
cagaaaccag ccttcttaga agggaggggg gggaaagtgt gaatgagaag ttgggggcgg 2460
agcgcgcggg ggaggggccg ctgccaggaa cgctcggcca aggctggcgc cgcgcccgcc 2520
ggtcgggcag cctcgcgccg cgctttgtct ccctctcggt gagtctcggc gggtcctgga 2580
ggccccagct ccaagcccgc cctccgcagc ccctccctcg ccctccgcgc acagcctttc 2640
agtgcagagt agtgactaaa cattacaaga agaccggccg ggcagttcca ggaatcgggg 2700
ggcggggcgt actggccggg taaatacccg cgcgcgcggc ctccgaggcg gctctaactc 2760
tgactctaca ctcgcccgct cctacgaccg ctgtctctcc gggctcccgg acgccccctt 2820
cccggcccag ctctccgtcg aggtccctcg cccaggtcct ttg 2863

<210> 3
<211> 3686
<212> DNA
<213> Mus musculus

<220>
<221> promoter
<222> (-2759)...(927)

<400> 3
ccctgctggc cctgcagagg gaaggctctc ctaagcatca tctatcagaa cgtcttcaaa 60
aaaaaaaaaa aaaaaaaaaa agcctctcca gccaggctag ctctaacacc atttcttccc 120
cttccctct ctcaaattca cttatctttt ttttttttt ttttttgga tttttgagac 180
agggtttctc tgtatagccc tggttgtcct ggaactcact ttgtacacca gcctggcctc 240
gaactcagag atccacctgc ctctgcctcc tgagtgctgg gattaaaggc gtgcgccacc 300
acgcccggct aaattcactt atctatttaa tgtatatagg gtataggctg cccttgaact 360
cacaaagatc tgcttgcttt gcttctggaa tactaaaggt gtgtgctacc atcacaggga 420
ccaagattta ttttaattct gtatatgtgt gtgtgtgtgt gtgtgtatgg ggggtgcaca 480
tgagtacaga ttcccttgga ggcctggggt ggcttaggac tggggttaca acagttgtga 540
cccatcctac ataggtcctg gcaccaacac ccccccccc ccccccccgtc ttccagaagt 600
gcagcaggtg ttcttaactg ctgagccagc aatccagccc ctgacttccc tctcttactt 660
aagaagctat cacagtgtct cactgggtca caatcatgac tagtccttgc tcatggccca 720
cagcctcttc cccactgtgg gttttgcccc gcagctctgc cgccccagcg ctgcacccga 780
ggcctgacag agccaggcac gaagtcaggg tttgtggaat ggatgaatga acttgactcg 840
tggcagagca ttgtaattta caaagcactt tcccatccat taactccagg gctatttcct 900
aagagtcctc cctgtcctcc actgccctcg gctcagaggc atacggtcaa ggcagtggct 960
ggggaacact ccctgaatga gatcaaggag ggcttgttca cagagaaagg gagaatccat 1020
ttggggagcc tgagagtgac tcgaaggcaa ggactgggcc tcacctgtgg gatctccatc 1080
tgtgagcatc cgctcatcag accagtgtga gatattttaa ataaggcccc taagcctctt 1140
gactactgga attgccaggg gcgggggaca gatgggcacc catccctatt taacagataa 1200
caagactgag tccagagagg cagtgcacct gccctggtct ctcttagttc ctcagcatca 1260
gtggagcaga ttggacacag tgggccagaa gggaagcagg cagccctccc tcctagccca 1320
agctactctg tgtagtcagt ttgccctcct actggtgtta caagaagcct gtggtatcca 1380
agagggcagg tcagaaagcc cactgagagc agacactgtg tgtcacttag ctggttctca 1440
ggtggctgcc acttcctgct gcctgttgca aaactcgaca ctaggccttt atagatactc 1500
acgtgaccag gagtaaacaa cctttcacct caatcacctg ctcttatcaa tactccctct 1560
ccaccccacc atcgggaaag ttcagacacc ttaaaacgta gaggcaagag agggtccatt 1620
ctgacacctc agcgactttc aggcagtggc tgaacccgtt acaacgctct gtggacagtc 1680
ctcctagtcg acattccttc tcaggtttga ccctgtcctg ggaagtgagg cttctctctc 1740
tgggttcccc actcctgttc ttgaataagg agccccacaa cctcttattc tctctataca 1800
gagcctggga aacagcaaaa ctcggctcgc ctacaagact ccagcgcgcc ctctggtgga 1860
ctcgggggac gagcatggga tgagggtttc tttcctctag ctcccccacc gcgccgagag 1920
tacctgggcg gacccacagt tcgccacgca ggttgggagg cccagatgag tgataaggta 1980

gtagttagct gctcctccca ccccactccc caaaggacat cagcacccac gtctgtcacc 2040
gaagaaccag gcaatgggcg gatgagctga ggccaggtag ctgcttctaa gtcagtgtct 2100
cctccacttc tggatctcac agcttcatct tttggacctg tctacaggta aatgtcgcgc 2160
atccccctcc tccacttcct aggtccccag tgggctggtg gctgaatggt cctacgtccc 2220
ttttggttgg cacgggatgc ttggaactgt acatgaggac ctcggggtgg cctgggtgca 2280
gagggagggg agcgtccccg cggggatcaa aagaaaggga aggggtgcca ggagggagcc 2340
tctcccggct ggcctcctag aactgcccgc gcgctcccat cgcgacgccc ccgcctctgc 2400
cagaaaccag ccttcttaga agggaggggg gggaaagtgt gaatgagaag ttgggggcgg 2460
agcgcgcggg ggaggggccg ctgccaggaa cgctcggcca aggctggcgc cgcgcccgcc 2520
ggtcgggcag cctcgcgccg cgctttgtct ccctctcggt gagtctcggc gggtcctgga 2580
ggccccagct ccaagcccgc cctccgcagc ccctccctcg ccctccgcgc acagcctttc 2640
agtgcagagt agtgactaaa cattacaaga agaccggccg ggcagttcca ggaatcgggg 2700
ggcggggcgt actggccggg taaatacccg cgcgcgcggc ctccgaggcg gctctaactc 2760
tgactctaca ctcgccccgct cctacgaccg ctgtctctcc gggctcccgg acgccccctt 2820
cccggcccag ctctccgtcg aggtccctcg cccaggtcct ttgcctgatt cgcccaggag 2880
tgcgcctcat cggcccgggg agcagcgaag ccagaggggg cgcacgcacg gggagccccc 2940
ttgtagactt cacggctgcc aacatctggg cgcagcgcga gccactgctg ggcgccgcct 3000
cgcctcgggg accataggag gcgcagcccc aaggccggag atttcgcttc gggactaggt 3060
aggaaggagg ggcgcggtgt ggggaagggt gggggcatcg gtccagctcg ggagcttttc 3120
ccggtttctc ctccccttcc cgggtcattc ccggtaggga ggggacgagg caggggggcag 3180
agcggatgag aaccgaagat ccctgattcc cgtcatactc agactggggc cctcgggttt 3240
ctcctgtccc ctctctcaca tatctcgggt ttggcacccc ccttttttcg ccctcgccac 3300
tgaggacacc ggactgagag gcgccctgag cgtccctagg gctcttgtgt ctctccccat 3360
cctggccgcg ctcctggaga cccaacttcc acgcgcgagt tttctctggg cgtcctccta 3420
gggcgggcag gggaagagac tgtctggggt tggccggcag tgaccgagga cagtcgagtt 3480
ccgcgaggtg gctgggcctg agacacggtc taaagcgggg caaaggggtg ccccgggcgc 3540
taggcggagg ctggagggcc gggcacgctg gagggttccg ggcactcacg cgcctcacgc 3600
tttgctctct gcagctcccc gggatgcggt agcggccgct gtgcggaggc cgcgaagcag 3660
ctgcagccac cgccgcgcag atccac 3686

<210> 4
<211> 2791
<212> DNA
<213> Mus musculus

<220>
<221> promoter
<222> (-1864)...(927)

<400> 4
ttcctaagag tcctccctgt cctccactgc cctcggctca gaggcatacg gtcaaggcag 60
tggctgggga acactccctg aatgagatca aggagggctt gttcacagag aaagggagaa 120
tccatttggg gagcctgaga gtgactcgaa ggcaaggact gggcctcacc tgtgggatct 180
ccatctgtga gcatccgctc atcagaccag tgtgagatat tttaaataag gcccctaagc 240
ctcttgacta ctggaattgc caggggcggg ggacagatgg gcacccatcc ctatttaaca 300
gataacaaga ctgagtccag agaggcagtg cacctgccct ggtctctctt agttcctcag 360
catcagtgga gcagattgga cacagtgggc cagaagggaa gcaggcagcc ctccctccta 420
gcccaagcta ctctgtgtag tcagtttgcc ctcctactgg tgttacaaga agcctgtggt 480
atccaagagg gcaggtcaga aagcccactg agagcagaca ctgtgtgtca cttagctggt 540
tctcaggtgg ctgccacttc ctgctgcctg ttgcaaaact cgacactagg cctttataga 600
tactcacgtg accaggagta aacaaccttt cacctcaatc acctgctctt atcaatactc 660
cctctccacc ccaccatcgg gaaagttcag acaccttaaa acgtagaggc aagagagggt 720
ccattctgac acctcagcga ctttcaggca gtggctgaac ccgttacaac gctctgtgga 780
cagtcctcct agtcgacatt ccttctcagg tttgaccctg tcctgggaag tgaggcttct 840
ctctctgggt tccccactcc tgttcttgaa taaggagccc cacaaccctct tattctctct 900

atacagagcc tgggaaacag caaaactcgg ctcgcctaca agactccagc gcgccctctg 960
gtggactcgg gggacgagca tgggatgagg gtttctttcc tctagctccc ccaccgcgcc 1020
gagagtacct gggcggaccc acagttcgcc acgcaggttg ggaggcccag atgagtgata 1080
aggtagtagt tagctgctcc tcccacccca ctccccaaag gacatcagca cccacgtctg 1140
tcaccgaaga accaggcaat gggcggatga gctgaggcca ggtagctgct tctaagtcag 1200
tgtctcctcc acttctggat ctcacagctt catcttttgg acctgtctac aggtaaatgt 1260
cgcgcatccc cctcctccac ttcctaggtc cccagtgggc tggtggctga atggtcctac 1320
gtccctttg gttggcacgg gatgcttgga actgtacatg aggacctcgg ggtggcctgg 1380
gtgcagaggg aggggagcgt ccccgcgggg atcaaaagaa agggaagggg tgccaggagg 1440
gagcctctcc cggctggcct cctagaactg cccgcgcgct cccatcgcga cgcccccgcc 1500
tctgccagaa accagccttc ttagaaggga gggggggaa agtgtgaatg agaagttggg 1560
ggcggagcgc gcgggggagg ggccgctgcc aggaacgctc ggccaaggct ggcgccgcgc 1620
ccgccggtcg ggcagcctcg cgccgcgctt tgtctccctc tcggtgagtc tcggcgggtc 1680
ctggaggccc cagctccaag cccgccctcc gcagcccctc cctcgccctc cgcgcacagc 1740
ctttcagtgc agagtagtga ctaaacatta caagaagacc ggccgggcag ttccaggaat 1800
cgggggcgg ggcgtactgg ccgggtaaat acccgcgcgc gcggcctccg aggcggctct 1860
aactctgact ctacactcgc ccgctcctac gaccgctgtc tctccgggct cccggacgcc 1920
cccttcccgg cccagctctc cgtcgaggtc cctcgcccag gtcctttgcc tgattcgccc 1980
aggagtgcgc ctcatcggcc cggggagcag cgaagccaga gggggcgcac gcacggggag 2040
ccccttgta gacttcacgg ctgccaacat ctgggcgcag cgcgagccac tgctgggcgc 2100
cgcctcgcct cggggaccat aggaggcgca gccccaaggc cggagatttc gcttcgggac 2160
taggtaggaa ggaggggcgc ggtgtgggga agggtggggg catcggtcca gctcgggagc 2220
ttttcccggt ttctcctccc cttcccgggt cattcccggt agggagggga cgaggcaggg 2280
ggcagagcgg atgagaaccg aagatccctg attcccgtca tactcagact ggggccctcg 2340
ggtttctcct gtcccctctc tcacatatct cgggtttggc acccccctt tttcgccctc 2400
gccactgagg acaccggact gagaggcgcc ctgagcgtcc ctagggctct tgtgtctctc 2460
cccatcctgg ccgcgctcct ggagacccaa cttccacgcg cgagttttct ctgggcgtcc 2520
tcctagggcg ggcaggggaa gagactgtct ggggttggcc ggcagtgacc gaggacagtc 2580
gagttccgcg aggtggctgg gcctgagaca cggtctaaag cggggcaaag gggtgccccg 2640
ggcgctaggc ggaggctgga gggccgggca cgctggaggg ttccgggcac tcacgcgcct 2700
cacgctttgc tctctgcagc tccccgggat gcggtagcgg ccgctgtgcg gaggccgcga 2760
agcagctgca gccaccgccg cgcagatcca c 2791

<210> 5
<211> 1803
<212> DNA
<213> Mus musculus

<220>
<221> promoter
<222> (-857)...(927)

<400> 5
gggtttcttt cctctagctc ccccaccgcg ccgagagtac ctgggcggac ccacagttcg 60
ccacgcaggt tgggaggccc agatgagtga taaggtagta gttagctgct cctcccaccc 120
cactccccaa aggacatcag cacccacgtc tgtcaccgaa gaaccaggca atgggcggat 180
gagctgaggc caggtagctg cttctaagtc agtgtctcct ccacttctgg atctcacagc 240
ttcatctttt ggacctgtct acaggtaaat gtcgcgcatc cccctcctcc acttcctagg 300
tccccagtgg gctggtggct gaatggtcct acgtcccttt tggttggcac gggatgcttg 360
gaactgtaca tgaggacctc ggggtggcct gggtgcagag ggaggggagc gtccccgcgg 420
ggatcaaaag aaagggaagg ggtgccagga gggagcctct cccggctggc ctcctagaac 480
tgcccgcgcg ctcccatcgc gacgcccccg cctctgccag aaaccagcct tcttagaagg 540
gaggggggg aaagtgtgaa tgagaagttg ggggcggagc gcgcgggga ggggccgctg 600
ccaggaacgc tcggccaagg ctggcgccgc gcccgccggt cgggcagcct cgcgccgcgc 660
tttgtctccc tctcggtgag tctcggcggg tcctggaggc cccagctcca agcccgccct 720

ccgcagcccc tccctcgccc tccgcgcaca gcctttcagt gcagagtagt gactaaacat 780
tacaagaaga ccggccgggc agttccagga atcggggggc ggggcgtact ggccgggtaa 840
atacccgcgc gcgcggcctc cgaggcggct ctaactctga ctctacactc gcccgctcct 900
acgaccgctg tctctccggg ctcccggacg ccccccttcc ggcccagctc tccgtcgagg 960
tccctcgccc aggtcctttg cctgattcgc ccaggagtgc gcctcatcgg cccggggagc 1020
agcgaagcca gagggggcgc acgcacgggg agccccctttg tagacttcac ggctgccaac 1080
atctgggcgc agcgcgagcc actgctgggc gccgcctcgc ctcggggacc ataggaggcg 1140
cagccccaag gccggagatt tcgcttcggg actaggtagg aaggaggggc gcggtgtggg 1200
gaagggtggg ggcatcggtc cagctcggga gcttttcccg gtttctcctc cccttcccgg 1260
gtcattcccg gtagggaggg gacgaggcag ggggcagagc ggatgagaac cgaagatccc 1320
tgattcccgt catactcaga ctggggccct cgggtttctc ctgtcccctc tctcacatat 1380
ctcgggtttg gcaccccccc ttttcgccc tcgccactga ggacaccgga ctgagaggcg 1440
ccctgagcgt ccctagggct cttgtgtctc tccccatcct ggccgcgctc ctggagaccc 1500
aacttccacg cgcgagtttt ctctgggcgt cctcctaggg cgggcagggg aagagactgt 1560
ctggggttgg ccggcagtga ccgaggacag tcgagttccg cgaggtggct gggcctgaga 1620
cacggtctaa agcggggcaa aggggtgccc cgggcgctag gcggaggctg gagggccggg 1680
cacgctggag ggttccgggc actcacgcgc ctcacgcttt gctctctgca gctccccggg 1740
atgcggtagc ggccgctgtg cggaggccgc gaagcagctg cagccaccgc cgcgcagatc 1800
cac 1803

<210> 6
<211> 990
<212> DNA
<213> Mus musculus

<220>
<221> promoter
<222> (-63)...(927)

<400> 6
gggggcgggg gcgtactggc cgggtaaata cccgcgcgcg cggcctccga ggcggctcta 60
actctgactc tacactcgcc cgctcctacg accgctgtct ctccgggctc ccggacgccc 120
ccttccccggc ccagctctcc gtcgaggtcc ctcgcccagg tcctttgcct gattcgccca 180
ggagtgcgcc tcatcggccc ggggagcagc gaagccagag ggggcgcacg cacggggagc 240
ccctttgtag acttcacggc tgccaacatc tgggcgcagc gcgagccact gctgggcgcc 300
gcctcgcctc ggggaccata ggaggcgcag ccccaaggcc ggagatttcg cttcgggact 360
aggtaggaag gaggggcgcg gtgtggggaa gggtgggggc atcggtccag ctcgggagct 420
tttcccggtt tctcctcccc ttcccgggtc attcccggta gggaggggac gaggcagggg 480
gcagagcgga tgagaaccga agatccctga ttcccgtcat actcagactg gggccctcgg 540
gtttctcctg tcccctctct cacatatctc gggtttggca ccccccttt ttcgccctcg 600
ccactgagga caccggactg agaggcgccc tgagcgtccc tagggctctt gtgtctctcc 660
ccatcctggc cgcgctcctg gagacccaac ttccacgcgc gagttttctc tgggcgtcct 720
cctagggcgg gcaggggaag agactgtctg gggttggccg gcagtgaccg aggacagtcg 780
agttccgcga ggtggctggg cctgagacac ggtctaaagc ggggcaaagg ggtgccccgg 840
gcgctaggcg gaggctggag ggccgggcac gctggagggt tccgggcact cacgcgcctc 900
acgctttgct ctctgcagct ccccgggatg cggtagcggc cgctgtgcgg aggccgcgaa 960
gcagctgcag ccaccgccgc gcagatccac 990

<210> 7
<211> 1024
<212> DNA
<213> Mus musculus

<220>
<221> promoter

<222> (-97)...(927)

<400> 7
ttacaagaag accggccggg cagttccagg aatcgggggg cggggcgtac tggccgggta 60
aatacccgcg cgcgcggcct ccgaggcggc tctaactctg actctacact cgcccgctcc 120
tacgaccgct gtctctccgg gctcccggac gcccccttcc cggcccagct ctccgtcgag 180
gtccctcgcc caggtccttt gcctgattcg cccaggagtg cgcctcatcg gcccggggag 240
cagcgaagcc agagggggcg cacgcacggg gagccccttt gtagacttca cggctgccaa 300
catctgggcg cagcgcgagc cactgctggg cgccgcctcg cctcggggac cataggaggc 360
gcagccccaa ggccggagat ttcgcttcgg gactaggtag gaaggagggg cgcggtgtgg 420
ggaagggtgg gggcatcggt ccagctcggg agcttttccc ggtttctcct ccccttcccg 480
ggtcattccc ggtagggagg ggacgaggca gggggcagag cggatgagaa ccgaagatcc 540
ctgattcccg tcatactcag actggggccc tcgggtttct cctgtcccct ctctcacata 600
tctcgggttt ggcacccccc ttttttcgcc ctcgccactg aggacaccgg actgagaggc 660
gccctgagcg tccctagggc tcttgtgtct ctccccatcc tggccgcgct cctggagacc 720
caacttccac gcgcgagttt tctctgggcg tcctcctagg gcgggcaggg gaagagactg 780
tctggggttg gccggcagtg accgaggaca gtcgagttcc gcgaggtggc tgggcctgag 840
acacggtcta aagcggggca aaggggtgcc ccgggcgcta ggcggaggct ggagggccgg 900
gcacgctgga gggttccggg cactcacgcg cctcacgctt tgctctctgc agctccccgg 960
gatgcggtag cggccgctgt gcggaggccg cgaagcagct gcagccaccg ccgcgcagat 1020
ccac 1024

<210> 8
<211> 201
<212> DNA
<213> Mus musculus

<220>
<221> promoter
<222> (-97)...(104)

<400> 8
ttacaagaag accggccggg cagttccagg aatcgggggg cggggcgtac tggccgggta 60
aatacccgcg cgcgcggcct ccgaggcggc tctaactctg actctacact cgcccgctcc 120
tacgaccgct gtctctccgg gctcccggac gcccccttcc cggcccagct ctccgtcgag 180
gtccctcgcc caggtccttt g 201

<210> 9
<211> 1014
<212> DNA
<213> Mus musculus

<220>
<221> promoter
<222> (-87)...(927)

<400> 9
accggccggg cagttccagg aatcgggggg cggggcgtac tggccgggta aatacccgcg 60
cgcgcggcct ccgaggcggc tctaactctg actctacact cgcccgctcc tacgaccgct 120
gtctctccgg gctcccggac gcccccttcc cggcccagct ctccgtcgag gtccctcgcc 180
caggtccttt gcctgattcg cccaggagtg cgcctcatcg gcccggggag cagcgaagcc 240
agagggggcg cacgcacggg gagccccttt gtagacttca cggctgccaa catctgggcg 300
cagcgcgagc cactgctggg cgccgcctcg cctcggggac cataggaggc gcagccccaa 360
ggccggagat ttcgcttcgg gactaggtag gaaggagggg cgcggtgtgg ggaagggtgg 420
gggcatcggt ccagctcggg agcttttccc ggtttctcct ccccttcccg ggtcattccc 480

ggtagggagg ggacgaggca gggggcagag cggatgagaa ccgaagatcc ctgattcccg 540
tcatactcag actggggccc tcgggtttct cctgtccect ctctcacata tctcgggttt 600
ggcacccccc ttttttcgcc ctcgccactg aggacaccgg actgagaggc gccctgagcg 660
tccctagggc tcttgtgtct ctccccatcc tggccgcgct cctggagacc caacttccac 720
gcgcgagttt tctctgggcg tcctcctagg gcgggcaggg gaagagactg tctggggttg 780
gccggcagtg accgaggaca gtcgagttcc gcgaggtggc tgggcctgag acacggtcta 840
aagcggggca aaggggtgcc ccgggcgcta ggcggaggct ggagggccgg gcacgctgga 900
gggttccggg cactcacgcg cctcacgctt tgctctctgc agctccccgg gatgcggtag 960
cggccgctgt gcggaggccg cgaagcagct gcagccaccg ccgcgcagat ccac 1014

<210> 10
<211> 191
<212> DNA
<213> Mus musculus

<220>
<221> promoter
<222> (-87)...(104)

<400> 10
accggccggg cagttccagg aatcgggggg cggggcgtac tggccgggta aatacccgcg 60
cgcgcggcct ccgaggcggc tctaactctg actctacact cgcccgctcc tacgaccgct 120
gtctctccgg gctcccggac gccccccttcc cggcccagct ctccgtcgag gtcccctcgcc 180
caggtccttt g 191

<210> 11
<211> 433
<212> DNA
<213> Mus musculus

<220>
<221> promoter
<222> (-275)...(158)

<400> 11
caggaacgct cggccaaggc tggcgccgcg cccgccggtc gggcagcctc gcgccgcgct 60
ttgtctccct ctcggtgagt ctcggcgggt cctggaggcc ccagctccaa gcccgccctc 120
cgcagccccct ccctcgccct ccgcgcacag cctttcagtg cagagtagtg actaaacatt 180
acaagaagac cggccgggca gttccaggaa tcgggggggcg gggcgtactg gccgggtaaa 240
tacccgcgcg cgcggcctcc gaggcggctc taactctgac tctacactcg cccgctccta 300
cgaccgctgt ctctccgggc tcccggacgc ccccttcccg gcccagctct ccgtcgaggt 360
ccctcgccca ggtcctttgc ctgattcgcc caggagtgcg cctcatcggc ccggggagca 420
gcgaagccag agg 433

<210> 12
<211> 1088
<212> DNA
<213> Mus musculus

<220>
<221> promoter
<222> (-161)...(927)

<400> 12
gccctccgca gcccctccct cgccctccgc gcacagcctt tcagtgcaga gtagtgacta 60

aacattacaa gaagaccggc cgggcagttc caggaatcgg ggggcggggc gtactggccg 120
ggtaaatacc cgcgcgcgcg gcctccgagg cggctctaac tctgactcta cactcgcccg 180
ctcctacgac cgctgtctct ccgggctccc ggacgccccc ttcccggccc agctctccgt 240
cgaggtccct cgcccaggtc ctttgcctga ttcgcccagg agtgcgcctc atcggcccgg 300
ggagcagcga agccagaggg ggcgcacgca cggggagccc ctttgtagac ttcacggctg 360
ccaacatctg ggcgcagcgc gagccactgc tgggcgccgc ctcgcctcgg ggaccatagg 420
aggcgcagcc ccaaggccgg agatttcgct tcgggactag gtaggaagga ggggcgcggt 480
gtggggaagg gtgggggcat cggtccagct cgggagcttt tcccggtttc tcctcccctt 540
cccgggtcat tcccggtagg gaggggacga ggcagggggc agagcggatg agaaccgaag 600
atccctgatt cccgtcatac tcagactggg gccctcgggt ttctcctgtc ccctctctca 660
catatctcgg gtttggcacc ccccttttt cgccctcgcc actgaggaca ccggactgag 720
aggcgccctg agcgtcccta gggctcttgt gtctctcccc atcctggccg cgctcctgga 780
gacccaactt ccacgcgcga gttttctctg ggcgtcctcc tagggcgggc aggggaagag 840
actgtctggg gttggccggc agtgaccgag gacagtcgag ttccgcgagg tggctgggcc 900
tgagacacgg tctaaagcgg ggcaaagggg tgccccgggc gctaggcgga ggctggaggg 960
ccgggcacgc tggagggttc cgggcactca cgcgcctcac gctttgctct ctgcagctcc 1020
ccgggatgcg gtagcggccg ctgtgcggag gccgcgaagc agctgcagcc accgccgcgc 1080
agatccac 1088

<210> 13
<211> 9
<212> DNA
<213> Mus musculus

<220>
<221> promoter
<222> (-74)...(-66)
<223> STAT-BINDING SITE AT -74 TO -66

<221> promoter
<222> (0)...(0)

<221> mutation
<222> (0)...(0)
<223> STAT-BINDING SITE AT -74 TO 66

<400> 13
ttccaggaa 9

<210> 14
<211> 9
<212> DNA
<213> Mus musculus

<220>
<221> mutation
<222> (-74)...(66)

<400> 14
atcgacgat 9

<210> 15
<211> 27
<212> DNA

<213> Artificial Sequence

<220>
<223> PRIMER

<400> 15
cagtagaatc cgctctcctg cagcttg 27

<210> 16
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> PRIMER

<221> promoter
<222> (0)...(0)

<221> promoter
<222> (0)...(0)

<221> promoter
<222> (0)...(0)

<221> promoter
<222> (0)...(0)

<221> promoter
<222> (0)...(0)

<221> promoter
<222> (0)...(0)

<400> 16
ctcgcttttg gagctgaagg tcttgag 27

<210> 17
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> PRIMER

<400> 17
cttcctacct agtcccgaag cgaaatc 27

<210> 18
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> PRIMER

<400> 18
cagatgttgg cagccgtgaa gtctac 26

<210> 19
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> PRIMER

<400> 19
gcgggcgagt gtagagtcag agttagag 28

<210> 20
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> PRIMER

<400> 20
cgattcctgg aactgcccgg ccggtcttc 29

<210> 21
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> PRIMER

<400> 21
ctcagtgggc tttctgacct gccctcttg 29

<210> 22
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> PRIMER

<400> 22
gactacacag agtagcttgg gctaggag 28

<210> 23
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> SENSE PRIMER

<400> 23
catcgcgacg cccccgcctc t 21

<210> 24
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> PRIMER

<400> 24
gaaacccgag ggccccagtc tg 22

<210> 25
<211> 21
<212> DNA
<213> Mus musculus

<220>
<221> promoter
<222> (-77)...(-57)

<400> 25
cagttccagg aatcgggggg c 21

<210> 26
<211> 21
<212> DNA
<213> Mus musculus

<220>
<221> promoter
<222> (0)...(0)
<223> MUTATIONS AT POSITIONS -74, -71, -69, & -66

<400> 26
cagatcgacg attcgggggg c 21

<210> 27
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> OLIGONUCLEOTIDE

<400> 27
gatcgaactg accgcccgcc gcccgt 26

<210> 28
<211> 30
<212> DNA
<213> Artificial Sequence

INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/US 00/40151

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N15/11 C07K14/47 A61K38/17 A61K48/00
A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A01K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! EBI; ACC.NO.: AF117732, 12 March 1999 (1999-03-12) AUERNHAMMER ET AL.: "Mus musculus suppressor of cytokine signalling-3 (Socs3) gene, partial cds." XP002150722	1-11
Y	the whole document	1-133
P,X	-& AUERNHAMMER ET AL.: "Autoregulation of pituitary corticotroph SOCS-3 expression: Characterization of the murine SOCS-3 promoter" PROC. NATL.ACAD SCI. USA, vol. 96, 8 June 1999 (1999-06-08), pages 6964-6969, XP002150720 page 6964 -page 6965; figures 1,3-5,7	1-11

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 October 2000	07/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	van Klompenburg, W

2

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/US 00/40151

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98 20023 A (INST MEDICAL W & E HALL ;VINEY ELIZABETH M (AU); STARR ROBYN (AU);) 14 May 1998 (1998-05-14) page 2, line 22 -page 3, line 10 page 4, line 4 - line 17 page 15, line 21 -page 16, line 7 page 44, line 10 - line 24 page 52, line 12 - line 16 page 55, line 20 - line 25 page 61, line 20 -page 62, line 2 claims 1-40; figure 9	1-133
X	DATABASE EMBL 'Online! EBI; ACC.NO.: AA124942, 26 November 1996 (1996-11-26) MARRA ET AL.: "The WashU-HHMI Mouse EST project" XP002150723 the whole document	1-11
X	DATABASE EMBL 'Online! EBI; ACC.NO.: W66798, 15 June 1996 (1996-06-15) MARRA ET AL.: "The WashU-HHMI Mouse ESTproject" XP002150724 the whole document	1-11
A	HEINRICH ET AL.: "Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway" BIOCHEMICAL JOURNAL, vol. 334, 1998, pages 297-314, XP002150721 cited in the application abstract page 305, right-hand column -page 306, left-hand column page 307, right-hand column figure 5 scheme 2	1-133

-/—

2

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	SEIJIRO MINAMOTO ET AL: "Cloning and functional analysis of new members of STAT induced STAT inhibitor (SSI) family: SSI.2 and SSI-3" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS,US,ACADEMIC PRESS INC. ORLANDO, FL, vol. 237, no. 1, 8 August 1997 (1997-08-08), pages 79-83, XP002095406 ISSN: 0006-291X cited in the application abstract figures 1,2,4	1-133

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/40151

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9820023 A	14-05-1998	AU 4694397 A	29-05-1998
		CN 1253565 A	17-05-2000
		EP 0948522 A	13-10-1999
		GB 2331753 A	02-06-1999
		NO 992116 A	29-06-1999